



CERTIFICATE OF TRANSLATION

I, Takeshi KOMATANI, a Patent Attorney, of Fifteenth Floor, Crystal Tower, 1-2-27 Shiromi, Chuo-ku, Osaka 540-6015, Japan HEREBY CERTIFY that I am acquainted with the English and Japanese languages and that I have read the attached English translation and found it to be a true and accurate translation of Japanese Patent Application No. 2003-092923 filed on March 28, 2003 in the name of INTELLECTUAL PROPERTY CONSULTING, INC.

Dated this 26th day of July, 2006.

A handwritten signature in cursive script, appearing to read "Takeshi Komatani", written over a horizontal line.

Takeshi KOMATANI



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(Translation)

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- 1 -

(Translation)

[Name of the Document] SPECIFICATION

[Title of the Invention] COMPOSITION AND METHOD FOR NERVE

5 REGENERATION

[Claims]

[Claim 1] A composition for regenerating nerves, comprising a Pep5 polypeptide.

10 [Claim 2] A composition according to claim 1, wherein the Pep5 polypeptide comprises:

(a) a polypeptide encoded by a nucleic acid sequence as set forth in SEQ ID NO. 1 or a fragment thereof;

15 (b) a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 2 or a fragment thereof;

(c) a variant polypeptide having an amino acid sequence as set forth in SEQ ID NO. 2 having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions, and deletions, wherein the variant polypeptide has a biological activity;

(d) a polypeptide encoded by a splice variant or an allelic variant of a base sequence as set forth in SEQ ID NO. 1;

25 (e) a species homolog polypeptide of a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 2; or

(f) a polypeptide consisting of an amino acid sequence having at least 70% identity to any one of the polypeptides of (a) to (e), wherein the polypeptide has a biological activity.

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[Claim 3] A composition according to claim 1, wherein the Pep5 polypeptide comprises the whole amino acid sequence as set forth in SEQ ID NO. 2.

[Claim 4] A composition according to claim 1, wherein the nerve includes spinal cord injury, cerebrovascular disorder, or brain injury.

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[Claim 5] A composition for regenerating nerves, comprising a nucleic acid molecule encoding a Pep5 polypeptide.

[Claim 6] A composition according to claim 5, wherein the
10 nucleic acid molecule encoding the Pep5 polypeptide comprises:

(a) a polynucleotide having a base sequence as set forth in SEQ ID NO. 1 or a fragment of the sequence thereof;

(b) a polynucleotide encoding an amino acid sequence as set forth in SEQ ID NO. 2 or a fragment thereof;

15 (c) a polynucleotide encoding a variant polypeptide having the amino acid sequence as set forth in SEQ ID NO. 2 having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions, and deletions, wherein the variant polypeptide has a biological
20 activity;

(d) a polynucleotide which is a splice variant or an allelic variant of a base sequence as set forth in SEQ ID NO. 1;

(e) a polynucleotide encoding a species homolog of a polypeptide consisting of an amino acid sequence as set forth
25 in SEQ ID NO. 2;

(f) a polynucleotide encoding a polypeptide hybridizable to any one of the polynucleotides of (a) to (e) under stringent conditions, wherein the polypeptide has a biological activity; or

30 (g) a polynucleotide consisting of a base sequence having at least 70% identity to any one of the polynucleotides of (a) to (e) or a complementary sequence thereof, wherein the polynucleotide encodes a polypeptide having a biological

activity.

[Claim 7] A composition according to claim 5, wherein the nucleic acid molecule encoding the Pep5 polypeptide comprises
5 the whole nucleotide sequence in the nucleic acid sequence as set forth in SEQ ID NO. 1.

[Claim 8] A composition according to claim 5, wherein the nerve includes spinal cord injury, cerebrovascular disorder,
10 or brain injury.

[Claim 9] A composition for regenerating nerves, comprising an agent capable of specifically interacting with a p75 polypeptide.
15

[Claim 10] A composition according to claim 9, wherein the p75 polypeptide comprises:

(a) a polypeptide encoded by a nucleotide having a nucleic acid sequence as set forth in SEQ ID NO. 3 or 16 or
20 a fragment thereof;

(b) a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 4 or 17 or a fragment thereof;

(c) a variant polypeptide having an amino acid sequence as set forth in SEQ ID NO. 4 or 17 having at least one mutation
25 selected from the group consisting of one or more amino acid substitutions, additions, and deletions, wherein the variant polypeptide has a biological activity;

(d) a polypeptide encoded by a splice variant or allelic variant of the base sequence as set forth in SEQ ID NO. 3 or
30 16;

(e) a species homolog polypeptide of a polypeptide having the amino acid sequence as set forth in SEQ ID NO. 4 or 17; or

(f) a polypeptide consisting of an amino acid sequence having at least 70% identity to the amino acid sequence of any one of the polypeptides of (a) to (e), wherein the polypeptide has a biological activity.

5

[Claim 11] A composition according to claim 9, wherein the p75 polypeptide comprises amino acids 273 to 427 or 274 to 425 of the amino acid sequence as set forth in SEQ ID NO. 4 or 17, respectively.

10

[Claim 12] A composition according to claim 9, wherein the nerve includes spinal cord injury, cerebrovascular disorder, or brain injury.

15 [Claim 13] A composition for regenerating nerves, comprising an agent specifically interacting with a nucleic acid molecule encoding a p75 polypeptide.

[Claim 14] A composition according to claim 13, wherein a
20 nucleic acid molecule encoding the p75 polypeptide comprises a polynucleotide selected from the group consisting of:

(a) a polynucleotide having a base sequence as set forth in SEQ ID NO. 3 or 16 or a fragment sequence thereof;

(b) a polynucleotide encoding an amino acid sequence
25 as set forth in SEQ ID NO. 4 or 17 or a fragment thereof;

(c) a polynucleotide encoding a variant polypeptide having the amino acid sequence as set forth in SEQ ID NO. 4 or 17 having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions,
30 and deletions, wherein the variant polypeptide has a biological activity;

(d) a polynucleotide which is a splice variant or allelic variant of a nucleotide of the base sequence as set

forth in SEQ ID NO. 3 or 16;

(e) a polynucleotide encoding a species homolog of a polypeptide consisting of the amino acid sequence as set forth in SEQ ID NO. 4 or 17;

5 (f) a polynucleotide hybridizable to any one of the polynucleotides of (a) to (e) under stringent conditions, wherein the polynucleotide encodes a polypeptide having a biological activity; or

10 (g) a polynucleotide consisting of a base sequence having at least 70% identity to any one of the polynucleotides of (a) to (e) or a complementary sequence thereof, wherein the polynucleotide encodes a polypeptide having a biological activity.

15 [Claim 15] A composition according to claim 13, wherein the nucleic acid molecule encoding the p75 polynucleotide comprises nucleotides 1110 to 1283 or 1113 to 1277 of the nucleic acid sequence as set forth in SEQ ID NO. 3 or 16, respectively.

20 [Claim 16] A composition according to claim 13, wherein the nerve includes spinal cord injury, cerebrovascular disorder, or brain injury.

[Claim 17] A composition for regenerating nerves, comprising a p75 extracellular domain polypeptide.

[Claim 18] A composition according to claim 17, wherein the p75 extracellular domain comprises:

30 (a) a polypeptide encoded by nucleotides 198 to 863 or 201 to 866 of a nucleic acid sequence as set forth in SEQ ID NO. 3 or 16, respectively, or a fragment thereof;

(b) a polypeptide having amino acids 29 to 250 or 30 to 251 of an amino acid sequence as set forth in SEQ ID NO. 4

or 17, respectively, or a fragment thereof;

(c) a variant polypeptide having amino acids 29 to 250 or 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4 or 17, respectively, having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions, and deletions, wherein the variant polypeptide has a biological activity;

(d) a polypeptide encoded by a sequence of a splice variant or allelic variant of nucleotides 198 to 863 or 201 to 866 of the base sequence as set forth in SEQ ID NO. 3 or 16, respectively;

(e) a species homolog polypeptide of a polypeptide having amino acids 29 to 250 or 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4 or 17, respectively;

(f) a polypeptide consisting of an amino acid sequence having at least 70% identity to any one of the polypeptides of (a) to (e), wherein the polypeptide has a biological activity.

[Claim 19] A composition according to claim 17, wherein the p75 extracellular domain polypeptide comprises amino acids 29 to 250 or 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4 or 17, respectively.

[Claim 20] A composition according to claim 17, wherein the nerve includes spinal cord injury, cerebrovascular disorder, or brain injury.

[Claim 21] A composition according to claim 17, wherein the p75 extracellular domain polypeptide is soluble.

[Claim 22] A composition for regenerating nerves, com-

prising a nucleic acid molecule encoding the p75 extracellular domain polypeptide.

[Claim 23] A composition according to claim 22, wherein the
5 nucleic acid molecule encoding the p75 extracellular domain polypeptide is a polynucleotide selected from the group consisting of:

(a) a polynucleotide having nucleotides 198 to 863 or
201 to 866 of a base sequence as set forth in SEQ ID NO. 3
10 or 16, respectively, or a fragment thereof;

(b) a polynucleotide encoding amino acids 29 to 250
or 30 to 251 of an amino acid sequence as set forth in SEQ
ID NO. 4 or 17, respectively, or a fragment thereof;

(c) a polynucleotide encoding a variant polypeptide
15 having amino acids 29 to 250 or 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4 or 17, respectively, having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions, and deletions, wherein the variant polypeptide has a biological
20 activity;

(d) a polynucleotide which is a splice variant or allelic variant of nucleotides 198 to 863 or 201 to 866 of the base sequence as set forth in SEQ ID NO. 3 or 16, respectively;

(e) a polynucleotide encoding a species homolog of a
25 polypeptide consisting of amino acid 29 to 250 or 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4 or 17, respectively;

(f) a polynucleotide hybridizable to any one of the
30 polynucleotides of (a) to (e) under stringent conditions, wherein the polynucleotide encodes a polypeptide having a biological activity; or

(g) a polynucleotide consisting of a base sequence

having at least 70% identity to any one of the polynucleotides of (a) to (e) or a complementary sequence thereof, wherein the polypeptide has a biological activity.

5 [Claim 24] A composition according to claim 22, wherein the nucleic acid molecule encoding the p75 extracellular domain polypeptide comprises nucleotides 198 to 863 or 201 to 866 of the nucleic acid sequence as set forth in SEQ ID NO. 3 or 16, respectively.

10

[Claim 25] A composition according to claim 22, wherein the nerve includes spinal cord injury, cerebrovascular disorder, or brain injury.

15 [Claim 26] A composition according to claim 22, wherein the p75 extracellular domain polypeptide is soluble.

[Claim 27] A composition for regenerating nerves, comprising an agent interacting with a Rho GDI polypeptide.

20

[Claim 28] A composition according to claim 27, wherein the Rho GDI polypeptide comprises:

(a) a polypeptide encoded by a nucleotide of a nucleic acid sequence as set forth in SEQ ID NO. 5 or a fragment thereof;

25 (b) a polypeptide having an amino acid sequence SEQ ID NO. 6 or a fragment thereof;

(c) a variant polypeptide having the amino acid sequence as set forth in SEQ ID NO. 6 having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions, and deletions, wherein the variant peptide has a biological activity;

30

(d) a polypeptide encoded by a splice variant or allelic variant of the base sequence as set forth in SEQ ID NO. 5;

(e) a species homolog polypeptide of a polypeptide having the amino acid sequence as set forth in SEQ ID NO. 6; or

5 (f) a polypeptide consisting of an amino acid sequence having at least 70% identity to any one of the polypeptides of (a) to (e), wherein the polypeptide has a biological activity.

[Claim 29] A composition according to claim 27, wherein the
10 Rho GDI polypeptide comprises the entire amino acid sequence as set forth in SEQ ID NO. 6.

[Claim 30] A composition according to claim 27, wherein the
15 nerve includes spinal cord injury, cerebrovascular disorder, or brain injury.

[Claim 31] A composition for regenerating nerves, comprising an agent specifically interacting with a nucleic acid molecule encoding a Rho GDI polypeptide.

20 [Claim 32] A composition according to claim 31, wherein the nucleic acid molecule encoding the Rho GDI polypeptide comprises a polynucleotide selected from the group consisting of:

25 (a) a polynucleotide having a nucleotide of the base sequence as set forth in SEQ ID NO. 5 or a fragment sequence thereof;

(b) a polynucleotide encoding an amino acid of an amino acid sequence as set forth in SEQ ID NO. 6 or a fragment thereof;

30 (c) a polynucleotide encoding a variant polypeptide having the amino acid of the amino acid sequence as set forth in SEQ ID NO. 6 having at least one mutation selected from the group consisting of one or more amino acid substitutions,

additions, and deletions, wherein the variant polypeptide has a biological activity;

(d) a polynucleotide which is a splice variant or allelic variant of a nucleotide of the base sequence as set forth in SEQ ID NO. 5;

(e) a polynucleotide encoding a species homolog of a polypeptide consisting of the amino acid sequence as set forth in SEQ ID NO. 6;

(f) a polynucleotide hybridizable to any one of the polynucleotides of (a) to (e) under stringent conditions, wherein the polynucleotide encodes a polypeptide having a biological activity; or

(g) a polynucleotide consisting of a base sequence having at least 70% identity to any one of the polynucleotides of (a) to (e) or a complementary sequence thereof, and wherein the polynucleotide encodes a polypeptide having a biological activity.

[Claim 33] A composition according to claim 31, wherein the Rho GDI comprises the entire nucleic acid sequence as set forth in SEQ ID NO. 5.

[Claim 34] A composition according to claim 31, wherein the nerve includes spinal cord injury, cerebrovascular disorder, or brain injury.

[Claim 35] A pharmaceutical composition according to any one of claims 1, 5, 9, 13, 17, 22, 27 and 31, wherein the nerve regeneration is due to disruption of neurite outgrowth inhibition.

[Claim 36] A method for regenerating nerves, comprising the step of providing a composition comprising at least one

molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, Rho GDI polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide to the nerve in an amount effective for regeneration.

[Claim 37] A method according to claim 36, wherein the nerve regeneration is due to disruption of neurite outgrowth inhibition.

[Claim 38] A composition for diagnosis, prophylaxis, treatment or prognosis of neurological diseases, disorders or conditions, comprising at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, a Rho GDI polypeptide, and a nucleic acid encoding the Rho GDI polypeptide in an amount effective for diagnosis, prophylaxis, treatment or prognosis.

[Claim 39] A method for diagnosis, prophylaxis, treatment or prognosis of neurological diseases, disorders or conditions, comprising the step of providing a composition comprising at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75

polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, a Rho GDI polypeptide, and
5 a nucleic acid encoding the Rho GDI polypeptide to the nerves, in an amount effective for diagnosis, prophylaxis, treatment or prognosis.

[Claim 40] A composition for constructing a network of
10 neurons, comprising at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75
15 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, a Rho GDI polypeptide, and a nucleic acid encoding the Rho GDI polypeptide.

[Claim 41] A method for constructing a network of neurons, comprising the step of: providing a composition comprising at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75
20 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, a Rho GDI polypeptide, and
25 a nucleic acid encoding the Rho GDI polypeptide to the nerves.

30

[Claim 42] A kit for treatment of neurological diseases, comprising: (A) a cell population regenerated with a composition comprising at least one molecule selected from

the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, a Rho GDI polypeptide, and a nucleic acid encoding the Rho GDI polypeptide; and

(B) a container for preserving the cell population.

[Claim 43] A method for treating neurological diseases, comprising the steps of:

(a) providing a cell population regenerated with a composition comprising at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, Rho GDI polypeptide, and a nucleic acid encoding the Rho GDI polypeptide; and

(b) transplanting the cell population to a patient.

[Claim 44] A screening method for identifying an agent which induces nerve regeneration, comprising the steps of:

(a) contacting a first polypeptide having an amino acid sequence having at least 70% homology to SEQ ID NO. 4 or 17 or a fragment thereof and a second polypeptide having an amino acid sequence having at least 70% homology to SEQ ID NO. 6 or a fragment thereof, in the presence of a test agent; and

(b) comparing a level of binding between the first polypeptide and the second polypeptide with a level of binding

in the absence of the test agent,

wherein the test agent is identified as an agent for
regenerating nerves when the binding in the presence of the
test agent is reduced as compared to that in the absence of
5 the test agent.

[Claim 45] A modulating agent, identified by a method
according to claim 44.

10 [Claim 46] A pharmaceutical composition, comprising a
modulating agent according to claim 45.

[Claim 47] A method for prophylaxis or treatment of
neurological diseases, disorders or conditions, comprising
15 the step of: administering a pharmaceutical composition
according to claim 46 to a subject.

[Claim 48] A vector, comprising at least one nucleic acid
molecule selected from the group consisting of a nucleic acid
20 molecule encoding a Pep5 polypeptide, a nucleic acid molecule
encoding a p75 polypeptide, and a nucleic acid molecule
encoding a Rho GDI polypeptide, wherein the at least one nucleic
acid molecule has a sequence comprising an introduced sequence
different from a sequence of a wild type.

25 [Claim 49] A cell, comprising a vector according to claim 48.

[Claim 50] A tissue, comprising a vector according to
claim 48.

30 [Claim 51] An organ, comprising a vector according to
claim 48.

[Claim 52] An organism, comprising a vector according to claim 48.

[Claim 53] A nerve-modified transgenic animal, transformed
5 with a vector according to claim 48.

[Claim 54] A nerve-modified knockout animal, wherein a
nucleic acid molecule selected from the group consisting of
a nucleic acid molecule encoding a Pep5 polypeptide, a nucleic
10 acid molecule encoding a p75 polypeptide, and a nucleic acid
encoding a Rho GDI polypeptide is deleted.

[Detailed Description of the Invention]

15 [0001]

[Field of the Invention]

The present invention relates to a pharmaceutical
composition and method for treating neurological diseases,
and a pharmaceutical composition and method for regenerating
20 nerves. Specifically, the present invention relates to a
pharmaceutical composition and method for treating neu-
rological diseases by disrupting inhibition of neurite
outgrowth.

25 [0002]

[Background Art]

The neurotrophin receptor (p75^{NTR}) mediates sur-
prisingly diverse biological effects (e. g., cell death,
Schwann cell migration, modulation of the synaptic
30 transmission, and functional regulation of sensory neurons
and calcium currents)(e. g., see Non-patent Document 1).
Recent work also implicates p75^{NTR} in the regulation of axon
elongation. Nerve growth factor (NGF) stimulates neurite

outgrowth from embryonic rat hippocampal neurons and chick ciliary neurons, which express only p75^{NTR} for NGF receptors (e. g., see Non-patent Document 2). These effects can be accounted for the modulation of Rho activity by p75^{NTR}. Rho is a low molecular GTPase that regulates the state of actin polymerization. In its active GTP-bound form, Rho rigidifies the actin cytoskeleton, thereby inhibiting axonal elongation and mediating growth cone collapse (e. g., see Non-patent Document 3 and 4). Neurotrophin binding to p75^{NTR} inactivates RhoA in HN10e cells as well as cerebellar neurons, whereas the over-expression of RhoA in the transfected 293 cells results in the activation of RhoA, suggesting that p75^{NTR} elicits bi-directional signals (e. g., see Non-patent Document 2). Indeed, subsequent study shows that myelin-associated glycoprotein (MAG), a glycoprotein derived from myelin, activates RhoA by a p75^{NTR}-dependent mechanism, thus inhibiting neurite outgrowth from postnatal sensory neurons and cerebellar neurons (e. g., see Non-patent Document 5). Furthermore, Nogo and oligodendrocyte myelin glycoprotein (OMgp), the other myelin-derived inhibitors of the neurite outgrowth, act on neurons via p75^{NTR} (e. g., see Non-patent Document 6). p75^{NTR} in complex with the Nogo receptor is suggested to form a receptor for all the myelin-derived inhibitors found so far (e. g., see Non-patent Document 6 and 7). However, precise mechanism of the regulation of Rho activity by p75^{NTR} remained to be elucidated.

[0003]

RhoA has been shown to interact with p75^{NTR} by the yeast two-hybrid system and co-immunoprecipitation (e. g., see Non-patent Document 2). As only the wild type of RhoA, which is predominantly in a GDP-bound form, but not the constitutive active form of RhoA, interacts dependent on a direct in-

teraction of RhoA and p75^{NTR}. Rho proteins in the GDP-bound form interact with Rho GDP dissociation inhibitor (Rho GDI), which plays a role in inhibiting nucleotide dissociation as well as the shuttling of Rho proteins between the cytoplasm and membranes (e. g., see Non-patent Document 8). Rho GDI prevents Rho family proteins from being converted to the active, GTP-bound form that is translocated to the membrane. In addition, after the active forms of Rho proteins are converted to the inactive forms at the membrane, Rho GDI forms a complex with them and translocates them to the cytosol. The Rho GDI family comprises at least three isoforms: Rho GDI α , Rho GDI β and Rho GDI γ . Rho GDI α is ubiquitously expressed and binds to all of the Rho family proteins thus far examined, whereas Rho GDI β and Rho GDI γ show unique tissue expression patterns and their substrate specificities have not been exactly determined.

[0004]

[Non-patent Document 1]

Dechant, G. & Barde, Y. A., Nat Neurosci. 5, 1131-1136 (2002)

[Non-patent Document 2]

Yamashita, T., Tucker, K. L. & Barde, Y. A., Neuron 24, 585-593 (1999)

[Non-patent Document 3]

Davies, A. M., Curr. Biol. 10, R198-200 (2000)

[Non-patent Document 4]

Schmidt, A. & Hall, A., Genes Dev. 16, 1587-1609 (2002)

[Non-patent Document 5]

Yamashita, T., Higuchi, H. & Tohyama, M., J. Cell Biol. 157, 565-570 (2002)

5 [Non-patent Document 6]

Wang, K. C. & Kim, J. A., Sivasankaran, R., Segal, R. & He, Z., Nature 420, 74-78 (2002)

[Non-patent Document 7]

10 Wong, S. T. et al., Nat Neurosci. 5, 1302-1308 (2002)

[Non-patent Document 8]

Sasaki, T. & Takai, Y., Biochem Biophys Res Commun. 245, 641-645 (1998)

15

[0005]

[Problems to be Solved by the Invention]

Considering the above-described discussion, an object of the present invention is to elucidate the relationship
20 between p75^{NTR}, which is involved in inhibition of neurite outgrowth, and agents capable of interacting therewith, thereby leading to regeneration of nerves and further treating neurological diseases based on the nerve regeneration.

25 [0006]

[Means for Solving the Problems]

The present inventors has solved the above-described problem in part by completely uncovering the signal transduction pathway via p75.

30

[0007]

The present inventors report the precise mechanism of the regulation of Rho activity by p75^{NTR}. Interestingly,

p75NTR shows activity of displacing the GDP-bound form of RhoA from GDI α . A peptide (Pep5), that was shown to specifically associate with p75^{NTR}, efficiently inhibits the signal mediated by p75^{NTR}, and may be a useful therapeutic agent in reversing
5 the growth inhibition elicited by myelin-derived inhibitors.

[0008]

The neurotrophin receptor p75NTR is involved in the regulation of axonal elongation by neurotrophins as well as
10 several myelin components (including myelin-associated glycoprotein, Nogo and oligodendrocyte myelin glycoprotein). neurite outgrowth by inhibiting Rho activity, whereas myelin-derived proteins activate RhoA, both through a p75NTR-dependent mechanism. Here, the present inventors show
15 that direct interaction of the Rho GDP dissociation inhibitor with p75NTR initiates the activation of RhoA. The interaction of p75NTR with Rho GDI is strengthened by myelin-associated glycoprotein or Nogo. p75NTR facilitates the release of prenylated RhoA from Rho GDP dissociation inhibitor. The
20 peptide ligand that was shown to be associated with the fifth of the six α -helices of p75NTR inhibits the interaction between Rho GDP dissociation inhibitor and p75NTR, thus silencing the action mediated by p75NTR. This peptide has potential as a therapeutic agent against the inhibitory cues that contribute
25 to the lack of regeneration of the central nervous system, i.e., an agent extinguishing the interaction between p75NTR and Rho GDI has the therapeutic potential for spinal cord injury, Alzheimer, cerebral infarction, cerebral hemorrhage, brain injury, and the like.

30

[0009]

Accordingly, the present invention provides the following.

[0010]

(1) A composition for regenerating nerves, comprising a Pep5 polypeptide.

5

[0011]

(2) A composition according to item 1, wherein the Pep5 polypeptide comprises:

10 (a) a polypeptide encoded by a nucleic acid sequence as set forth in SEQ ID NO. 1 or a fragment thereof;

(b) a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 2 or a fragment thereof;

15 (c) a variant polypeptide having an amino acid sequence as set forth in SEQ ID NO. 2 having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions, and deletions, wherein the variant polypeptide has a biological activity;

(d) a polypeptide encoded by a splice variant or an allelic variant of a base sequence as set forth in SEQ ID NO. 1;

20 (e) a species homolog polypeptide of a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 2; or

(f) a polypeptide consisting of an amino acid sequence having at least 70% identity to any one of the polypeptides of (a) to (e), wherein the polypeptide has a biological activity.

25

[0012]

30 (3) A composition according to item 1, wherein the Pep5 polypeptide comprises the whole amino acid sequence as set forth in SEQ ID NO. 2.

[0013]

(4) A composition according to item 1, wherein the nerve includes spinal cord injury, cerebrovascular disorder, or brain injury.

5 [0014]

(5) A composition for regenerating nerves, comprising a nucleic acid molecule encoding a Pep5 polypeptide.

[0015]

10 (6) A composition according to item 5, wherein the nucleic acid molecule encoding the Pep5 polypeptide comprises:

(a) a polynucleotide having a base sequence as set forth in SEQ ID NO. 1 or a fragment thereof;

15 (b) a polynucleotide encoding an amino acid sequence as set forth in SEQ ID NO. 2 or a fragment thereof;

(c) a polynucleotide encoding a variant polypeptide having the amino acid sequence as set forth in SEQ ID NO. 2 having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions, and
20 deletions, wherein the variant polypeptide has a biological activity;

(d) a polynucleotide which is a splice variant or an allelic variant of a base sequence as set forth in SEQ ID NO. 1;

25 (e) a polynucleotide encoding a species homolog of a polypeptide consisting of an amino acid sequence as set forth in SEQ ID NO. 2;

(f) a polynucleotide encoding a polypeptide hybridizable to any one of the polynucleotides of (a) to (e) under stringent conditions, wherein the polypeptide has a
30 biological activity; or

(g) a polynucleotide consisting of a base sequence having at least 70% identity to any one of the polynucleotides of (a) to (e) or a complementary sequence thereof, wherein

the polynucleotide encodes a polypeptide having a biological activity.

[0016]

- 5 (7) A composition according to item 5, wherein the nucleic acid molecule encoding the Pep5 polypeptide comprises the whole nucleotide sequence in the nucleic acid sequence as set forth in SEQ ID NO. 1.

10 [0017]

(8) A composition according to item 5, wherein the nerve includes spinal cord injury, cerebrovascular disorder, or brain injury.

15 [0018]

(9) A composition for regenerating nerves, comprising an agent capable of specifically interacting with a p75 polypeptide.

[0019]

- 20 (10) A composition according to item 9, wherein the p75 polypeptide comprises:

(a) a polypeptide encoded by a nucleotide of a nucleic acid sequence as set forth in SEQ ID NO. 3 or 16 or a fragment thereof;

- 25 (b) a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 4 or 17 or a fragment thereof;

(c) a variant polypeptide having the amino acid sequence as set forth in SEQ ID NO. 4 or 17 having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions, and deletions, wherein
30 the variant polypeptide has a biological activity;

(d) a polypeptide encoded by a splice variant or allelic variant of the base sequence as set forth in SEQ ID NO. 3 or

16;

(e) a species homolog polypeptide of a polypeptide having the amino acid sequence as set forth in SEQ ID NO. 4 or 17; or

5 (f) a polypeptide consisting of an amino acid sequence having at least 70% identity to the amino acid sequence of any one of the polypeptides of (a) to (e), wherein the polypeptide has a biological activity.

10 [0020]

(11) A composition according to item 9, wherein the p75 polypeptide comprises amino acids 273 to 427 or 274 to 425 of the amino acid sequence as set forth in SEQ ID NO. 4 or 17, respectively.

15

[0021]

(12) A composition according to item 9, wherein the nerve includes spinal cord injury, cerebrovascular disorder, or brain injury.

20

[0022]

(13) A composition for regenerating nerves, comprising an agent specifically interacting with a nucleic acid molecule encoding a p75 polypeptide.

25

[0023]

(14) A composition according to item 13, wherein a nucleic acid molecule encoding the p75 polypeptide comprises a polynucleotide selected from the group consisting of:

30

(a) a polynucleotide having a base sequence as set forth in SEQ ID NO. 3 or 16 or a fragment sequence thereof;

(b) a polynucleotide encoding an amino acid sequence as set forth in SEQ ID NO. 4 or 17 or a fragment thereof;

(c) a polynucleotide encoding a variant polypeptide having the amino acid sequence as set forth in SEQ ID NO. 4 or 17 having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions, and deletions, wherein the variant polypeptide has a biological activity;

(d) a polynucleotide which is a splice variant or allelic variant of a nucleotide of the base sequence as set forth in SEQ ID NO. 3 or 16;

(e) a polynucleotide encoding a species homolog of a polypeptide consisting of the amino acid sequence as set forth in SEQ ID NO. 4 or 17;

(f) a polynucleotide hybridizable to any one of the polynucleotides of (a) to (e) under stringent conditions, wherein the polynucleotide encodes a polypeptide having a biological activity; or

(g) a polynucleotide consisting of a base sequence having at least 70% identity to any one of the polynucleotides of (a) to (e) or a complementary sequence thereof, wherein the polynucleotide encodes a polypeptide having a biological activity.

[0024]

(15) A composition according to item 13, wherein the nucleic acid molecule encoding the p75 polynucleotide comprises nucleotides 1110 to 1283 or 1113 to 1277 of the nucleic acid sequence as set forth in SEQ ID NO. 3 or 16, respectively.

[0025]

(16) A composition according to item 13, wherein the nerve includes spinal cord injury, cerebrovascular disorder, or brain injury.

[0026]

(17) A composition for regenerating nerves, comprising a p75 extracellular domain polypeptide.

5 [0027]

(18) A composition according to item 17, wherein the p75 extracellular domain comprises:

(a) a polypeptide encoded by nucleotides 198 to 863 or 201 to 866 of a nucleic acid sequence as set forth in SEQ
10 ID NO. 3 or 16, respectively, or a fragment thereof;

(b) a polypeptide having amino acids 29 to 250 or 30 to 251 of an amino acid sequence as set forth in SEQ ID NO. 4 or 17, respectively, or a fragment thereof;

(c) a variant polypeptide having amino acids 29 to 250
15 or 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4 or 17, respectively, having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions, and deletions, wherein the variant polypeptide has a biological activity;

20 (d) a polypeptide encoded by a sequence of a splice variant or allelic variant of nucleotides 198 to 863 or 201 to 866 of the base sequence as set forth in SEQ ID NO. 3 or 16, respectively;

(e) a species homolog polypeptide of a polypeptide
25 having amino acids 29 to 250 or 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4 or 17, respectively;
or

(f) a polypeptide consisting of an amino acid sequence
30 having at least 70% identity to any one of the polypeptides of (a) to (e), wherein the polypeptide has a biological activity.

[0028]

(19) A composition according to item 17, wherein the p75 extracellular domain polypeptide comprises amino acids 29 to 250 or 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4 or 17, respectively.

5

[0029]

(20) A composition according to item 17, wherein the nerve includes spinal cord injury, cerebrovascular disorder, or brain injury.

10

[0030]

(21) A composition according to item 17, wherein the p75 extracellular domain polypeptide is soluble.

15 [0031]

(22) A composition for regenerating nerves, comprising a nucleic acid molecule encoding the p75 extracellular domain polypeptide.

20 [0032]

(23) A composition according to item 22, wherein the nucleic acid molecule encoding the p75 extracellular domain polypeptide is a polynucleotide selected from the group consisting of:

25 (a) a polynucleotide having nucleotides 198 to 863 or 201 to 866 of a base sequence as set forth in SEQ ID NO. 3 or 16, respectively, or a fragment thereof;

(b) a polynucleotide encoding amino acids 29 to 250 or 30 to 251 of an amino acid sequence as set forth in SEQ
30 ID NO. 4 or 17, respectively, or a fragment thereof;

(c) a polynucleotide encoding a variant polypeptide having amino acids 29 to 250 or 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4 or 17, respectively,

having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions, and deletions, wherein the variant polypeptide has a biological activity;

5 (d) a polynucleotide which is a splice variant or allelic variant of nucleotides 198 to 863 or 201 to 866 of the base sequence as set forth in SEQ ID NO. 3 or 16, respectively;

10 (e) a polynucleotide encoding a species homolog of a polypeptide consisting of amino acid 29 to 250 or 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4 or 17, respectively;

(f) a polynucleotide hybridizable to any one of the polynucleotides of (a) to (e) under stringent conditions, 15 wherein the polynucleotide encodes a polypeptide having a biological activity; or

(g) a polynucleotide consisting of a base sequence having at least 70% identity to any one of the polynucleotides of (a) to (e) or a complementary sequence thereof, wherein 20 the polypeptide has a biological activity.

[0033]

(24) A composition according to item 22, wherein the nucleic acid molecule encoding the p75 extracellular domain 25 polypeptide comprises nucleotides 198 to 863 or 201 to 866 of the nucleic acid sequence as set forth in SEQ ID NO. 3 or 16, respectively.

[0034]

30 (25) A composition according to item 22, wherein the nerve includes spinal cord injury, cerebrovascular disorder, or brain injury.

[0035]

(26) A composition according to item 22, wherein the p75 extracellular domain polypeptide is soluble.

5 [0036]

(27) A composition for regenerating nerves, comprising an agent interacting with a Rho GDI polypeptide.

[0037]

10 (28) A composition according to item 27, wherein the Rho GDI polypeptide comprises:

(a) a polypeptide encoded by a nucleotide of a nucleic acid sequence as set forth in SEQ ID NO. 5 or a fragment thereof;

15 (b) a polypeptide having an amino acid sequence SEQ ID NO. 6 or a fragment thereof;

(c) a variant polypeptide having the amino acid sequence as set forth in SEQ ID NO. 6 having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions, and deletions, wherein the variant peptide has a biological activity;

(d) a polypeptide encoded by a splice variant or allelic variant of the base sequence as set forth in SEQ ID NO. 5;

25 (e) a species homolog polypeptide of a polypeptide having the amino acid sequence as set forth in SEQ ID NO. 6; or

(f) a polypeptide consisting of an amino acid sequence having at least 70% identity to any one of the polypeptides of (a) to (e), wherein the polypeptide has a biological activity.

30

[0038]

(29) A composition according to item 27, wherein the Rho GDI polypeptide comprises the entire amino acid sequence as set

forth in SEQ ID NO. 6.

[0039]

(30) A composition according to item 27, wherein the nerve
5 includes spinal cord injury, cerebrovascular disorder, or
brain injury.

[0040]

(31) A composition for regenerating nerves, comprising an
10 agent specifically interacting with a nucleic acid molecule
encoding a Rho GDI polypeptide.

[0041]

(32) A composition according to item 31, wherein the nucleic
15 acid molecule encoding the Rho GDI polypeptide comprises a
polynucleotide selected from the group consisting of:

(a) a polynucleotide having the base sequence as set
forth in SEQ ID NO. 5 or a fragment sequence thereof;

(b) a polynucleotide encoding an amino acid of an amino
20 acid sequence as set forth in SEQ ID NO. 6 or a fragment thereof;

(c) a polynucleotide encoding a variant polypeptide
having the amino acid of the amino acid sequence as set forth
in SEQ ID NO. 6 having at least one mutation selected from
the group consisting of one or more amino acid substitutions,
25 additions, and deletions, wherein the variant polypeptide has
a biological activity;

(d) a polynucleotide which is a splice variant or
allelic variant of a nucleotide of the base sequence as set
forth in SEQ ID NO. 5;

(e) a polynucleotide encoding a species homolog of a
30 polypeptide consisting of the amino acid sequence as set forth
in SEQ ID NO. 6;

(f) a polynucleotide hybridizable to any one of the

polynucleotides of (a) to (e) under stringent conditions, wherein the polynucleotide encodes a polypeptide having a biological activity; or

5 (g) a polynucleotide consisting of a base sequence having at least 70% identity to any one of the polynucleotides of (a) to (e) or a complementary sequence thereof, and wherein the polynucleotide encodes a polypeptide having a biological activity.

10 [0042]

(33) A composition according to item 31, wherein the Rho GDI comprises the entire nucleic acid sequence as set forth in SEQ ID NO. 5.

15 [0043]

(34) A composition according to item 31, wherein the nerve includes spinal cord injury, cerebrovascular disorder, or brain injury.

20 [0044]

(35) A pharmaceutical composition according to any one of items 1, 5, 9, 13, 17, 22, 27 and 31, wherein the nerve regeneration is due to disruption of neurite outgrowth inhibition.

25

[0045]

(36) A method for regenerating nerves, comprising the step of providing a composition comprising at least one molecule selected from the group consisting of a Pep5 polypeptide, a
30 nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide,

a nucleic acid molecule encoding the p75 extracellular domain polypeptide, Rho GDI polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide to the nerve in an amount effective for regeneration.

5

[0046]

(37) A method according to item 36, wherein the nerve regeneration is due to disruption of neurite outgrowth inhibition.

10

[0047]

(38) A composition for diagnosis, prophylaxis, treatment or prognosis of neurological diseases, disorders or conditions, comprising at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, a Rho GDI polypeptide, and a nucleic acid encoding the Rho GDI polypeptide in an amount effective for diagnosis, prophylaxis, treatment or prognosis.

25

[0048]

(39) A method for diagnosis, prophylaxis, treatment or prognosis of neurological diseases, disorders or conditions, comprising the step of providing a composition comprising at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular

30

domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, a Rho GDI polypeptide, and a nucleic acid encoding the Rho GDI polypeptide to the nerves, in an amount effective for diagnosis, prophylaxis, treatment or prognosis.

[0049]

(40) A composition for constructing a network of neurons, comprising at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, a Rho GDI polypeptide, and a nucleic acid encoding the Rho GDI polypeptide.

[0050]

(41) A method for constructing a network of neurons, comprising the step of: providing a composition comprising at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, a Rho GDI polypeptide, and a nucleic acid encoding the Rho GDI polypeptide to the nerves.

[0051]

(42) A kit for treatment of neurological diseases, comprising:
(A) a cell population regenerated with a composition

comprising at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, a Rho GDI polypeptide, and a nucleic acid encoding the Rho GDI polypeptide; and

10 (B) a container for preserving the cell population.

[0052]

(43) A method for treating neurological diseases, comprising the steps of:

15 (a) providing a cell population regenerated with a composition comprising at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, Rho GDI polypeptide, and a nucleic acid encoding the Rho GDI polypeptide; and

25 (b) transplanting the cell population to a patient.

[0053]

(44) A screening method for identifying an agent which induces nerve regeneration, comprising the steps of:

30 (a) contacting a first polypeptide having an amino acid sequence having at least 70% homology to SEQ ID NO. 4 or 17 or a fragment thereof and a second polypeptide having an amino acid sequence having at least 70% homology to SEQ ID NO. 6

or a fragment thereof, in the presence of a test agent; and

(b) comparing a level of binding between the first polypeptide and the second polypeptide with a level of binding in the absence of the test agent,

5 wherein the test agent is identified as an agent for regenerating nerves when the binding in the presence of the test agent is reduced as compared to that in the absence of the test agent.

10 [0054]

(45) A modulating agent, identified by a method according to item 44.

[0055]

15 (46) A pharmaceutical composition, comprising a modulating agent according to item 45.

[0056]

20 (47) A method for prophylaxis or treatment of neurological diseases, disorders or conditions, comprising the step of: administering a pharmaceutical composition according to claim 46 to a subject.

[0057]

25 (48) A vector, comprising at least one nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a Pep5 polypeptide, a nucleic acid molecule encoding a p75 polypeptide, and a nucleic acid molecule encoding a Rho GDI polypeptide, wherein the at least one nucleic acid molecule
30 has a sequence comprising an introduced sequence different from a sequence of a wild type.

[0058]

(49) A cell, comprising a vector according to item 48.

[0059]

(50) A tissue, comprising a vector according to item 48.

5

[0060]

(51) An organ, comprising a vector according to item 48.

[0061]

10 (52) An organism, comprising a vector according to item 48.

[0062]

(53) A nerve-modified transgenic animal, transformed with a vector according to item 48.

15

[0063]

(54) A nerve-modified knockout animal, wherein a nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a Pep5 polypeptide, a nucleic acid molecule encoding a p75 polypeptide, and a nucleic acid encoding a Rho GDI polypeptide is deleted.

20

[0064]

[Embodiments of the Invention]

25 It should be understood throughout the present specification that expressions with singular forms include the concept of their plurality unless otherwise mentioned. It should be also understood that terms as used herein have definitions ordinarily used in the art unless otherwise
30 mentioned.

[0065]

(Definitions)

As used herein, "p75 signal transduction pathway" refers to a series of signal transduction pathways from activation of Rho by myelin-derived proteins via the p75 receptor on nerve membranes to inhibition of neurite outgrowth, i. e., a mechanism causing a phenomenon that once a central nerve axon is injured, the axon can no longer regenerate. Referring to FIG. 6, the p75 signal transduction pathway is a pathway in which when a myelin-derived protein acts on p75, Rho is activated via p75, so that neurite outgrowth is inhibited.

[0066]

As used herein, "Pep5" refers to a peptide which binds to the intracellular domain of p75 to inhibit activation of Rho by p75. Representatively, Peps has sequences as set forth in SEQ ID NO. 1 (degenerate nucleic acid sequence) and SEQ ID NO. 2 (amino acid sequence). Variants and fragments of Pep5 are also included within the definition of Pep5 as long as they have biological activity. Examples of the biological activity of Pep5 include, but are not limited to, blocking of neurite outgrowth inhibition by a myelin-derived protein. Such activity can be measured with a Rho activity assay which blocks activation of Rho by a myelin-derived protein, or the like.

[0067]

As used herein, "p75^{NTR}" refers to a neurotrophin receptor which is involved in the regulation of axonal elongation by a neurotrophin and several myelin components (including myelin-binding glycoprotein, Nogo, and oligodendrocyte myelin glycoprotein). The neurotrophin receptor p75 (p75^{NTR}) mediates surprisingly diverse biological effects (e. g., see Non-patent Document 1) (e.g., cell death, Schwann

cell migration, modulation of synaptic transmission, and functional regulation of sensory neurons and calcium currents). Recent work also implicates p75^{NTR} in the regulation of axon elongation.

5

[0068]

As used herein, "p75" is used interchangeably with p75^{NTR} to refer to a single transmembrane receptor which mediates signal transduction of a myelin-derived protein where a neurotrophin is a ligand. Representatively, p75 has sequences as set forth in SEQ ID NO. 3 or 16 (human or rat nucleic acid sequences, respectively) and SEQ ID NO. 4 or 17 (human or rat amino acid sequences, respectively), and their variants and fragments are also included within the definition of p75 as long as they have biological activity. Examples of the biological activity of p75 include, but are not limited to, promotion of neurite outgrowth by a neurotrophin. Such activity can be measured with an assay which blocks activation of Rho by a myelin-derived protein, or the like.

20

[0069]

As used herein, "p75 extracellular domain" refers to an extracellular portion of amino terminus of p75 which is a single transmembrane receptor present on cell membranes. The p75 extracellular domain representatively has sequences indicated by positions 1110-1283 of SEQ ID NO. 3 (human nucleic acid sequence) or positions 1113-1277 of SEQ ID NO. 16 (rat nucleic acid sequence) and positions 273-427 of SEQ ID NO. 4 (human amino acid sequence) or positions 274-425 of SEQ ID NO. 17 (rat amino acid sequence), and their variants and fragments are also included within the definition of the p75 extracellular domain as long as they have biological activity. Examples of the biological activity of the p75 extracellular

30

domain include, but are not limited to, blocking of neurite outgrowth inhibition by a myelin-derived protein. Such activity can be measured with an assay which blocks activation of Rho by a myelin-derived protein, or the like.

5

[0070]

The terms "Rho GDP release inhibiting protein" or "Rho GDI" are used interchangeably to refer to a protein which has a role in inhibition of nucleotide release and the shuttling of Rho proteins between cytoplasm and membrane (e. g., see Non-patent Document 8). Rho GDI prevents the Rho family proteins from being transformed into active GTP-bound forms which are translocated to membranes. After the Rho protein in the active form is transformed into an inactive form, Rho GDI and the Rho protein form a complex which is then translocated to the cytosol. The Rho GDI family includes at least three isoforms: Rho GDI α , Rho GDI β , and Rho GDI γ . Rho GDI α is ubiquitously expressed and binds to all Rho family proteins which have been heretofore studied. Rho GDI β and Rho GDI γ exhibit particular tissue expression patterns. Rho GDI representatively has sequences as set forth in SEQ ID NO. 5 (nucleic acid sequence) and SEQ ID NO. 6 (amino acid sequence), and their variants and fragments are also included within the definition of Rho GDI as long as they have biological activity. Examples of the biological activity of Rho GDI include, but are not limited to, binding to GDP-bound Rho. Such activity can be measured with an assay, such as a GDP-GTP exchange assay.

[0071]

As used herein, "MAG" is an abbreviation of "myelin-binding glycoprotein" to refer to a glycoprotein present on oligodendrocyte and Schwann cell membranes. MAG representatively has sequences as set forth in SEQ ID NO. 7

(nucleic acid sequence) and SEQ ID NO. 8 (amino acid sequence), and their variants and fragments are also included within the definition of MAG as long as they have biological activity. Examples of the biological activity of MAG include, but are not limited to, neurite outgrowth inhibition. Such activity can be measured with an assay which observes activation of Rho in nerve cells.

[0072]

As used herein, "Nogo" refers to a double transmembrane protein present on cell membranes of oligodendrocytes. Nogo representatively has sequences as set forth in SEQ ID NO. 9 (nucleic acid sequence) and SEQ ID NO. 10 (amino acid sequence), and their variants and fragments are also included within the definition of Nogo as long as they have biological activity. Examples of the biological activity of Nogo include, but are not limited to, inhibition of neurite outgrowth of nerve cells. Such activity can be measured with an assay which observes Rho activation in nerve cells, or the like.

[0073]

The term "Rho" refers to a low molecular weight GTPase which regulates the state of actin polymerization. In its active GTP-bound form, Rho hardens the actin cytoskeleton, thereby inhibiting axonal elongation and mediating destruction of growth cones (e. g., see Non-patent Documents 3 and 4). Rho representatively has sequences as set forth in SEQ ID NO. 11 (nucleic acid sequence) and SEQ ID NO. 12 (amino acid sequence) which are RhoA sequences described below. Their variants and fragments are also included within the definition of Rho as long as they have biological activity. Examples of the biological activity of Rho include, but are not limited to, control of neurite outgrowth. Such activity can be measured

by an assay, such as affinity precipitation using an effector protein, or the like.

[0074]

5 As used herein, "RhoA" refers to a molecule which is
a member of the Rho family. RhoA representatively has sequences
as set forth in SEQ ID NO. 11 (nucleic acid sequence) and SEQ
ID NO. 12 (amino acid sequence), and their variants and
10 fragments are also included within the definition of RhoA as
long as they have biological activity. Examples of the
biological activity of RhoA include, but are not limited to,
control of neurite outgrowth. Such activity can be measured
with an assay, such as affinity precipitation using an effector
15 protein.

[0075]

 As used herein, "GT1b" refers to a molecule which is
a type of ganglioside and is used in the same meaning as defined
in the art. Examples of the biological activity of GT1b include,
20 but are not limited to, binding to p75. Such activity can be
measured with an assay, such as a binding experiment to p75.

[0076]

 As used herein, "p21" refers to a molecule of cy-
25 clin-dependent protein kinase inhibitor, and also called as
WAF1 or Cip1. p21 representatively has sequences as set forth
in SEQ ID NO. 13 (nucleic acid sequence) and SEQ ID NO. 14
(amino acid sequence), and their variants and fragments are
also included within the definition of p21 as long as they
30 have biological activity. Examples of the biological
activity of p21 include, but are not limited to, cell cycle
arrest. Such activity can be measured with an assay, such as
molecular induction of nerve cells.

[0077]

The terms "silencing" and "silence" are used herein interchangeably to refer to disruption of the interaction
5 between p75^{NTR} and Rho GDI. The term "silencer" refers to an agent which disrupts the interaction between p75^{NTR} and Rho GDI.

[0078]

10 (Definition of Terms)

Hereinafter, the definitions of the terms as used herein are enumerated.

[00079]

15 The terms "protein", "polypeptide", "oligopeptide" and "peptide" as used herein have the same meaning and refer to an amino acid polymer having any length. This polymer may be a straight, branched or cyclic chain. An amino acid may be a naturally-occurring or nonnaturally-occurring amino acid,
20 or a variant amino acid. The term may include those assembled into a complex of a plurality of polypeptide chains. The term also includes a naturally-occurring or artificially modified amino acid polymer. Such modification includes, for example, disulfide bond formation, glycosylation, lipidation,
25 acetylation, phosphorylation, or any other manipulation or modification (e.g., conjugation with a labeling moiety). This definition encompasses a polypeptide containing one or more amino acid analog (including, e. g., nonnaturally-occurring amino acid, etc.), a peptide-like compound (e. g., peptoid),
30 and other variants known in the art, for example. Gene products of the present invention are ordinarily in the form of polypeptides. Such gene products of the present invention in the polypeptide form are useful for compositions of the present

invention for diagnosis, prophylaxis, treatment or prognosis.

[0080]

The terms "polynucleotide", "oligonucleotide", and
5 "nucleic acid" as used herein have the same meaning and refer
to a nucleotide polymer having any length. This term also
includes an "oligonucleotide derivative" or a "polynucleotide
derivative". An "oligonucleotide derivative" or a
10 "polynucleotide derivative" includes a nucleotide derivative,
or refers to an oligonucleotide or a polynucleotide having
different linkages between nucleotides from typical linkages,
which are interchangeably used. Examples of such an
oligonucleotide specifically include
2'-O-methyl-ribonucleotide, an oligonucleotide derivative in
15 which a phosphodiester bond in an oligonucleotide is converted
to a phosphorothioate bond, an oligonucleotide derivative in
which a phosphodiester bond in an oligonucleotide is converted
to a N3'-P5' phosphoroamidate bond, an oligonucleotide
derivative in which a ribose and a phosphodiester bond in an
20 oligonucleotide are converted to a peptide-nucleic acid bond,
an oligonucleotide derivative in which uracil in an oli-
gonucleotide is substituted with C-5 propynyl uracil, an
oligonucleotide derivative in which uracil in an oli-
gonucleotide is substituted with C-5 thiazole uracil, an
25 oligonucleotide derivative in which cytosine in an oli-
gonucleotide is substituted with C-5 propynyl cytosine, an
oligonucleotide derivative in which cytosine in an oli-
gonucleotide is substituted with phenoxazine-modified
cytosine, an oligonucleotide derivative in which ribose in
30 DNA is substituted with 2'-O-propyl ribose, and an oli-
gonucleotide derivative in which ribose in an oligonucleotide
is substituted with 2'-methoxyethoxy ribose. Unless otherwise
indicated, a particular nucleic acid sequence also implicitly

encompasses conservatively modified variants thereof (e.g. degenerate codon substitutions) and complementary sequences and as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be produced by generating
5 sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzner et al., Nucleic Acid Res. 19:5081(1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); Rossolini et al., Mol. Cell. Probes. 8:91-98(1994)).
10 Genes of the present invention are ordinarily in the form of the above-described polynucleotides. Such genes or gene products of the present invention in the polynucleotide form are useful for compositions of the present invention for diagnosis, prophylaxis, treatment or prognosis.

15 [0081]

As used herein, "nucleic acid molecule" is also used interchangeably with nucleic acid, oligonucleotide and polynucleotide, including cDNA, mRNA, genomic DNA, and the like. As used herein, nucleic acid and nucleic acid molecule
20 may be included by the concept of the term "gene". A nucleic acid molecule encoding the sequence of a given gene includes "splice mutant (variant)". Similarly, a particular protein encoded by a nucleic acid encompasses any protein encoded by
25 a splice variant of that nucleic acid. "Splice mutants", as the name suggests, are products of alternative splicing of a gene. After transcription, an initial nucleic acid transcript may be spliced such that different (another) nucleic acid splice products encode different polypeptides. Mechanisms for
30 the production of splice mutants vary, but include alternative splicing of exons. Alternative polypeptides derived from the same nucleic acid by read-through transcription are also encompassed by this definition. Any products of a splicing

reaction, including recombinant forms of the splice products, are included in this definition. Therefore, the gene of the present invention may include the splice mutants herein.

5 [0082]

As used herein, "gene" refers to an agent defining a genetic trait. A gene is typically arranged in a given sequence on a chromosome. A gene which regulates the expression of a structural gene is called a regulatory gene (e. g., promoter).
10 Genes herein include structural genes and regulatory genes unless otherwise specified. Therefore, Pep5, p75, Rho GDI, MAG and p21 ordinarily include both the structural genes of the gene of the present invention and the regulatory sequences such as promoters for transcription and/or translation and
15 the like. In the present invention, it will be understood that in addition to structural genes, regulatory sequences for transcription and/or translation and the like are useful for nerve regeneration, and diagnosis, treatment, prophylaxis and prognosis for nerve diseases, and the like. As used herein,
20 "gene" may refer to "polynucleotide", "oligonucleotide", "nucleic acid", and "nucleic acid molecule" and/or "protein", "polypeptide", "oligopeptide" and "peptide". As used herein, "gene product" includes "polynucleotide", "oligonucleotide", "nucleic acid" and "nucleic acid molecule" and/or "protein",
25 "polypeptide", "oligopeptide" and "peptide", which are expressed by a gene. Those skilled in the art understand what a gene product is, according to the context.

[0083]

30 As used herein, "homology" of a gene (e.g., a nucleic acid sequence, an amino acid sequence, or the like) refers to the proportion of identity between two or more gene sequences. As used herein, the identity of a sequence (a nucleic acid

sequence, an amino acid sequence, or the like) refers to the proportion of the identical sequence (an individual nucleic acid, amino acid, or the like) between two or more comparable sequences. Therefore, the greater the homology between two
5 given genes, the greater the identity or similarity between their sequences. Whether or not two genes have homology may be checked by comparing their sequences directly or by a hybridization method under stringent conditions. When two gene sequences are directly compared with each other, these genes
10 have homology if the DNA sequences of the genes have representatively at least 50% identity, preferably at least 70% identity, more preferably at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% identity with each other. As used herein, "similarity" of a gene (e.g., a nucleic acid sequence, an amino
15 acid sequence, or the like) refers to the proportion of identity between two or more sequences when conservative substitution is regarded as positive (identical) in the above-described homology. Therefore, homology and similarity differ from each other in the presence of conservative substitutions. If no
20 conservative substitutions are present, homology and similarity have the same value.

[0084]

The similarity, identity and homology of amino acid
25 sequences and base sequences are herein compared using FASTA which is a sequence analyzing tool with default parameters.

[0085]

As used herein, "amino acid" may refer to a natu-
30 rally-occurring or nonnaturally-occurring amino acid as long as it satisfies the purpose of the present invention. The term "amino acid derivative" or "amino acid analog" refers to an amino acid which is different from a naturally-occurring amino

acid and has a function similar to that of the original amino acid. Such an amino acid derivative and amino acid analog are well known in the art. The term "naturally-occurring amino acid" refers to an L-isomer of a naturally-occurring amino acid. The naturally-occurring amino acids are glycine, alanine, valine, leucine, isoleucine, serine, methionine, threonine, phenylalanine, tyrosine, tryptophan, cysteine, proline, histidine, aspartic acid, asparagine, glutamic acid, glutamine, γ -carboxyglutamic acid, arginine, ornithine, and lysine. Unless otherwise indicated, all amino acids as used herein are L-isomers, although embodiments using D-amino acids are within the scope of the present invention. The term "nonnaturally-occurring amino acid" refers to an amino acid which is ordinarily not found in the nature. Examples of nonnaturally-occurring amino acids include norleucine, para-nitrophenylalanine, homophenylalanine, para-fluorophenylalanine, 3-amino-2-benzil propionic acid, D- or L-homoarginine, and D-phenylalanine. The term "amino acid analog" refers to a molecule having a physical property and/or function similar to that of amino acids, but not an amino acid. Examples of amino acid analogs include, for example, ethionine, canavanine, 2-methylglutamine, and the like. An amino acid mimic refers to a compound which has a structure different from that of the general chemical structure of amino acids but which functions in a manner similar to that of naturally-occurring amino acids.

[0086]

Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0087]

As used herein, the term "corresponding" amino acid refers to an amino acid in a given protein molecule or polypeptide molecule, which has, or is anticipated to have, activity similar to that of a predetermined amino acid in a protein or polypeptide as a reference for comparison. Particularly, in the case of enzyme molecules, the term refers to an amino acid which is present at a similar position in an active site and similarly contributes to catalytic activity. For example, in the case of antisense molecules, the term refers to a similar portion in an ortholog corresponding to a particular portion of the antisense molecule.

15 [0088]

As used herein, the term "corresponding" gene refers to a gene in a given species, which has, or is anticipated to have, a function similar to that of a predetermined gene in a species as a reference for comparison. When there are a plurality of genes having such a function, the term refers to a gene having the same evolutionary origin. Therefore, a gene corresponding to a given gene may be an ortholog of the given gene. Therefore, genes corresponding to mouse Pep5, PKC, p75, Rho GDI, MAG, and p21 can be found in other animals (human, rat, pig, cattle, and the like). Such a corresponding gene can be identified by a technique well known in the art. Therefore, for example, a corresponding gene in a given animal can be found by searching a sequence database of the animal (e.g., human, rat) using the sequence of a reference gene (e.g., mouse Pep5, PKC, p75, Rho GDI, MAG, p21 and the like) as a query sequence.

[0089]

As used herein, the term "nucleotide" may be either naturally-occurring or nonnaturally-occurring. The term "nucleotide derivative" or "nucleotide analog" refers to a nucleotide which is different from a naturally-occurring
5 nucleotide and has a function similar to that of the original nucleotide. Such a nucleotide derivative and nucleotide analog are well known in the art. Examples of such a nucleotide derivative and nucleotide analog include, but are not limited to, phosphorothioate, phosphoramidate, methylphosphonate,
10 chiral-methylphosphonate, 2-O-methyl ribonucleotide, and peptide-nucleic acid (PNA).

[0090]

As used herein, the term "fragment" refers to a
15 polypeptide or polynucleotide having a sequence length ranging from 1 to n-1 with respect to the full length of the reference polypeptide or polynucleotide (of length n). The length of the fragment can be appropriately changed depending on the purpose. For example, in the case of polypeptides, the lower
20 limit of the length of the fragment includes 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50 or more amino acids. Lengths represented by integers which are not herein specified (e.g., 11 and the like) may be appropriate as a lower limit. For example, in the case of polynucleotides, the lower limit of the length
25 of the fragment includes 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100 or more nucleotides. Lengths represented by integers which are not herein specified (e.g., 11 and the like) may be appropriate as a lower limit. As used herein, the length of polypeptides or polynucleotides can be represented by the
30 number of amino acids or nucleic acids, respectively as described above. However, the above-described numbers are not absolute. The above-described numbers as the upper or lower limit are intended to include some greater or smaller numbers

(or e.g. 10% greater or smaller), as long as it has the same function. For this purpose, "about" may be herein put ahead of the numbers. However, it should be understood that the interpretation of numbers is not affected by the presence or absence of "about" in the present specification. The length of a useful fragment may be determined depending on whether or not at least one function is maintained among the functions of a full-length protein which is a reference of the fragment.

10 [0091]

As used herein, the term "agent specifically interacting with" a biological agent such as a polynucleotide, a polypeptide or the like, refers to an agent which has an affinity to the biological agent, such as a polynucleotide, a polypeptide or the like, which is representatively higher than or equal to an affinity to other non-related biological agents, such as polynucleotides, polypeptides or the like (particularly, those with identity of less than 30%), and preferably significantly (e.g., statistically significantly) higher. Such an affinity can be measured with, for example, a hybridization assay, a binding assay, or the like. As used herein, the "agent" may be any substance or other element (e. g., energy such as light, radiation, heat, electricity, or the like) as long as the intended purpose can be achieved. Examples of such a substance include, but are not limited to, proteins, polypeptides, oligopeptides, peptides, polynucleotides, oligonucleotides, nucleotides, nucleic acids (including, e.g., DNA such as cDNA and genomic DNA, and RNA such as mRNA), polysaccharides, oligosaccharides, lipids, organic low molecules (e.g., hormones, ligands, information transfer substances, molecules synthesized by combinatorial chemistry, low molecules which may be used as a medicament_ (e. g., low molecular weight ligands and the like), and the like), and

combinations of these molecules. Examples of an agent specific to a polynucleotide include, but are not limited to, representatively, a polynucleotide having complementarity to the sequence of the polynucleotide with a predetermined sequence homology (e. g., 70% or more sequence identity), a polypeptide such as a transcriptional agent binding to a promoter region, and the like. Examples of an agent specific to a polypeptide include, but are not limited to, representatively, an antibody specifically directed to the polypeptide or derivatives or analogs thereof (e.g., single chain antibody), a specific ligand or receptor when the polypeptide is a receptor or ligand, a substrate when the polypeptide is an enzyme, and the like.

15 [0092]

As used herein, the term "organic low molecule" refers to an organic molecule having a relatively small molecular weight. Usually, the organic low molecule refers to a molecular weight of about 1,000 or less, or may refer to a molecular weight of more than 1,000. Organic low molecules can be ordinarily synthesized by methods known in the art or combinations thereof. These organic low molecules may be produced by organisms. Examples of the organic low molecule include, but are not limited to, hormones, ligands, information transfer substances, synthesized by combinatorial chemistry, low molecules which may be used as a medicament (e.g., low molecular weight ligands and the like), and the like.

[0093]

30 As used herein, the term "antibody" encompasses polyclonal antibodies, monoclonal antibodies, human antibodies, humanized antibodies, polyfunctional antibodies, chimeric antibodies, and anti-idiotypic antibodies, and

fragments thereof, e.g., F(ab')₂ and Fab fragments, and other conjugates produced by other recombinations. These antibodies may be fused with an enzyme, e.g., alkaline phosphatase, horseradish peroxidase, α-galactosidase, and the like via a covalent bond or by recombination.

[0094]

As used herein, the term "monoclonal antibody" refers to an antibody composition having a group of homologous antibodies. This term is not limited by the production manner thereof. This term encompasses all immunoglobulin molecules and Fab molecules, F(ab')₂ fragments, Fv fragments, and other molecules having an immunological binding property of the original monoclonal antibody molecule. Methods for producing polyclonal antibodies and monoclonal antibodies are well known in the art, and will be more sufficiently described below.

Monoclonal antibodies are prepared by using the standard technique well known in the art (e.g., Kohler and Milstein, Nature (1975) 256:495) or a modification thereof (e.g., Buck et al. (1982) In Vitro 18:377). Representatively, a mouse or rat is immunized with a protein bound to a protein carrier, and boosted. Subsequently, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate or well coated with a protein antigen. B-cells that express membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas. The hybridomas are used to produce monoclonal antibodies:

[0095]

As used herein, the term "antigen" refers to any substrate to which an antibody molecule may specifically bind. As used herein, the term "immunogen" refers to an antigen
5 capable of initiating activation of the antigen-specific immune response of a lymphocyte.

[0096]

As used herein, the term "single chain antibody" refers
10 to a single chain polypeptide formed by linking a heavy chain fragment and the light chain fragment of the Fv region via amino acid crosslinking.

[0097]

As used herein, the term "composite molecule" refers
15 to a molecule in which a plurality of molecules, such as polypeptides, polynucleotides, lipids, sugars, low molecules, and the like, are linked together. Examples of such a composite molecule include, but are not limited to, glycolipids,
20 glycopeptides, and the like. These composite molecules can be used herein as nucleic acid molecules encoding Pep5, p75, Rho GDI, MAG, p21 and, variants or fragments thereof, and the like, products thereof, GT1b, or the agent of the present invention as long as they have a function similar to that of
25 the nucleic acid molecules encoding Pep5, p75, Rho GDI, MAG, p21, and variants or fragments thereof, and the like, products thereof, GT1b, or the agent of the present invention.

[0098]

As used herein, the term "isolated" biological agent
30 (e. g., nucleic acid, protein, or the like) refers to a biological agent that is substantially separated or purified from other biological agents in cells of a naturally-occurring

organism (e. g., in the case of nucleic acids, agents other than nucleic acids and a nucleic acid having nucleic acid sequences other than an intended nucleic acid; and in the case of proteins, agents other than proteins and proteins having an amino acid sequence other than an intended protein). The "isolated" nucleic acid and protein include nucleic acids and proteins purified by a standard purification method. The isolated nucleic acids and proteins also include chemically synthesized nucleic acids and proteins.

[0099]

As used herein, the term "purified" biological agent (e. g., nucleic acids, proteins, and the like) refers to one from which at least a part of naturally accompanying agents is removed. Therefore, ordinarily, the purity of the biological agent of a purified biological agent is higher than the biological agent in a normal state (i. e., concentrated).

[0100]

As used herein, the terms "purified" and "isolated" mean that the same type of biological agent is present preferably at least 75% by weight, more preferably at least 85% by weight, even more preferably at least 95% by weight, and most preferably at least 98% by weight.

[0101]

As used herein, the term "expression" of a gene product, such as a gene, a polynucleotide, a polypeptide, or the like, indicates that the gene or the like is affected by a predetermined action in vivo to be changed into another form. Preferably, the term "expression" indicates that genes, polynucleotides, or the like are transcribed and translated into polypeptides. It is also one form of expression when genes

are transcribed to produce mRNA. More preferably, these polypeptides may have post-translational processing.

[0102]

5 Therefore, as used herein, the term "reduction" of
"expression" of a gene, a polynucleotide, a polypeptide, or
the like indicates that the amount of expression is sig-
nificantly reduced in the presence of the action of the agent
of the present invention as compared to when the action of
10 the agent is absent. Preferably, the reduction of expression
includes a reduction in the amount of expression of a
polypeptide (e. g., Pep5, p75, Rho GDI, MAG and p21). As used
herein, the term "increase" of "expression" of a gene, a
polynucleotide, a polypeptide, or the like indicates that the
15 amount of expression is significantly increased in the presence
of the action of the agent of the present invention as compared
to when the action of the agent is absent. Preferably, the
increase of expression includes an increase in the amount of
expression of a polypeptide (e.g., Pep5, p75, Rho GDI, MAG
20 and p21). As used herein, the term "induction" of "expression"
of a gene indicates that the amount of expression of the gene
is increased by applying a given agent to a given cell. Therefore,
the induction of expression includes allowing a gene to be
expressed when expression of the gene is not observed at all,
25 and increasing the amount of expression of the gene when
expression of the gene has been already observed. The increase
or reduction of these genes or gene products (polypeptides
or polynucleotides) may be useful in treatment embodiments,
prognosis embodiments or prophylaxis embodiments of the
30 present invention.

[0103]

As used herein, the term "specifically expressed" in

the case of genes indicates that a gene is expressed in a specific site in an organism or in a specific period of time at a level different from (preferably higher than) that in other sites or periods of time. The term "specifically expressed" may indicate that a gene is expressed only in a given site (specific site) or expressed in other sites. Preferably, the term "specifically expressed" indicates that a gene is expressed only in a given site. Therefore, according to an embodiment of the present invention, Pep5, p75, Rho GDI, MAG and p21 may be expressed specifically and locally in an affected portion (e.g., nerve).

[0104]

As used herein, term "biological activity" refers to activity possessed by an agent (e.g., a polynucleotide, a protein, etc.) within an organism, including activities exhibiting various functions (e.g., transcription promoting activity). For example, when two agents interact with each other (e.g., Pep5 and p75, p75 and Rho GDI, MAG and p75, GT1b and p75), the biological activity may be binding of the two molecules and a biological change due to the binding. For example, when one molecule is precipitated using antibodies, another molecule may also precipitate. Therefore, observation of such coprecipitation provides a determination method, for example. In addition, neurite outgrowth may be used as an indicator to infer that a given molecule is functionally associated with another molecule. Specifically, the term "biological activity" includes the observation that MAG, GT1b, p75, and Rho GDI inhibit neurite outgrowth in association with one another, while Pep5 and p21 block this action. For example, when a given agent is an enzyme, the biological activity thereof includes the enzymatic activity thereof. In another example, when a given agent is a ligand,

the biological activity thereof includes binding of the agent to a receptor for the ligand. Such biological activity can be measured with a technique well known in the art.

5 [0105]

As used herein, the term "antisense (activity)" refers to activity which permits specific suppression or reduction of expression of a target gene. The antisense activity is ordinarily achieved by a nucleic acid sequence having a length
10 of at least 8 contiguous nucleotides, which is complementary to the nucleic acid sequence of a target gene (e. g., Pep5, p75, Rho GDI, MAG p21 and the like). Such a nucleic acid sequence preferably has a length of at least 8 contiguous nucleotides, more preferably a length of at least 10 contiguous nucleotides,
15 and even more preferably a length of at least 11 contiguous nucleotides, a length of at least 12 contiguous nucleotides, a length of at least 13 contiguous nucleotides, a length of at least 14 contiguous nucleotides, a length of at least 15 contiguous nucleotides, a length of at least 20 contiguous
20 nucleotides, a length of at least 30 contiguous nucleotides, a length of at least 40 contiguous nucleotides, and a length of at least 50 contiguous nucleotides. These nucleic acid sequences include nucleic acid sequences having at least 70% homology thereto, more preferably at least 80%, even more
25 preferably at least 90%, and most preferably at least 95%. Such an antisense activity is preferably complementary to a 5' terminal sequence of the nucleic acid sequence of a target gene. Such an antisense nucleic acid sequence includes the above-described sequences having one or several, or at least
30 one, nucleotide substitutions, additions, and/or deletions.

[0106]

As used herein, the term "RNAi" is an abbreviation of

RNA interference and refers to a phenomenon that an agent for causing RNAi, such as double-stranded RNA (also called dsRNA), is introduced into cells and mRNA homologous thereto is specifically degraded, so that synthesis of gene products is suppressed, and a technique used for the phenomenon. As used herein, RNAi may have the same meaning as that of an agent which causes RNAi.

[0107]

As used herein, the term "an agent causing RNAi" refers to any agent capable of causing RNAi. As used herein, "an agent causing RNAi for a gene" indicates that the agent causes RNAi relating to the gene and the effect of RNAi is achieved (e.g., suppression of expression of the gene, and the like). Examples of such an agent causing RNAi include, but are not limited to, a sequence having at least about 70% homology to the nucleic acid sequence of a target gene or a sequence hybridizable under stringent conditions, RNA containing a double-stranded portion having a length of at least 10 nucleotides or variants thereof. Here, this agent may be preferably DNA containing a 3' protruding end, and more preferably the 3' protruding end has a length of 2 or more nucleotides (e. g., 2-4 nucleotides in length).

[0108]

As used herein, "polynucleotides hybridizing under stringent conditions" refers to conditions commonly used and well known in the art. Such a polynucleotide can be obtained by conducting colony hybridization, plaque hybridization, Southern blot hybridization, or the like using a polynucleotide selected from the polynucleotides of the present invention. Specifically, a filter on which DNA derived from a colony or plaque is immobilized is used to conduct hybridization at 65°C

in the presence of 0.7 to 1.0 M NaCl. Thereafter, a 0.1 to 2-fold concentration SSC (saline-sodium citrate) solution (1-fold concentration SSC solution is composed of 150 mM sodium chloride and 15 mM sodium citrate) is used to wash the filter at 65°C. Polynucleotides identified by this method are referred to as "polynucleotides hybridizing under stringent conditions". Hybridization can be conducted in accordance with a method described in, for example, Molecular Cloning 2nd ed., Current Protocols in Molecular Biology, Supplement 1-38, DNA Cloning 1: Core Techniques, A Practical Approach, Second Edition, Oxford University Press (1995), and the like. Here, sequences hybridizing under stringent conditions exclude, preferably, sequences containing only A or T. "Hybridizable polynucleotide" refers to a polynucleotide which can hybridize other polynucleotides under the above-described hybridization conditions. Specifically, the hybridizable polynucleotide includes at least a polynucleotide having a homology of at least 60% to the base sequence of DNA encoding a polypeptide having an amino acid sequence specifically herein disclosed, preferably a polynucleotide having a homology of at least 80%, and more preferably a polynucleotide having a homology of at least 95%.

[0109]

The term "highly stringent conditions" refers to those conditions that are designed to permit hybridization of DNA strands whose sequences are highly complementary, and to exclude hybridization of significantly mismatched DNAs. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of "highly stringent conditions" for hybridization and washing are 0.0015 M sodium chloride, 0.0015M sodium citrate at 65-68°C or 0.015M

sodium chloride, 0.0015M sodium citrate, and 50% formamide at 42°C. See Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory (Cold Spring Harbor, N.Y., 1989); and Anderson et al., Nucleic Acid Hybridization: a Practical Approach IV, IRL Press Limited (Oxford, England). Limited, Oxford, England. More stringent conditions (such as higher temperature, lower ionic strength, higher formamide, or other denaturing agents) may be used if necessary. Other agents may be included in the hybridization and washing buffers for the purpose of reducing non-specific and/or background hybridization. Examples are 0.1% bovine serum albumin, 0.1% polyvinylpyrrolidone, 0.1% sodium pyrophosphate, 0.1% sodium dodecylsulfate (NaDodSO₄ or SDS), Ficoll, Denhardt's solution, sonicated salmon sperm DNA (or another noncomplementary DNA), and dextran sulfate, although other suitable agents can also be used. The concentration and types of these additives can be changed without substantially affecting the stringency of the hybridization conditions. Hybridization experiments are ordinarily carried out at pH 6.8-7.4; however, at typical ionic strength conditions, the rate of hybridization is almost independent of pH. See Anderson et al., Nucleic Acid Hybridization: a Practical Approach Chapter 4, IRL Press Limited, (Oxford, England).

25 [0110]

Agents affecting the stability of DNA duplex include base composition, length, and degree of base pair mismatch. Hybridization conditions can be adjusted by those skilled in the art to accommodate these variables and allow DNAs of different sequence relatedness to form hybrids. The melting temperature of a perfectly matched DNA duplex can be estimated by the following equation:

$$T_m \quad (^\circ\text{C}) = 81.5 + 16.6 \quad (\log[\text{Na}^+]) + 0.41 \quad (\%G+C) - 600/N - 0.72$$

(%formamide)

where N is the length of the duplex formed, [Na+] is the molar concentration of the sodium ion in the hybridization or washing solution, % G+C is the percentage of (guanine+cytosine) bases in the hybrid. For imperfectly matched hybrids, the melting temperature is reduced by approximately 1°C for each 1% mismatch.

[0111]

The term "moderately stringent conditions" refers to conditions under which a DNA duplex with a greater degree of base pair mismatching than could occur under "highly stringent conditions" is able to form. Examples of typical "moderately stringent conditions" are 0.015M sodium chloride, 0.0015M sodium citrate at 50-65°C or 0.015M sodium chloride, 0.0015M sodium citrate, and 20% formamide at 37-50°C. By way of example, "moderately stringent" conditions of 50°C in 0.015M sodium ion will allow about a 21% mismatch.

[0112]

It will be appreciated by those skilled in the art that there is no absolute distinction between "highly stringent conditions" and "moderately stringent conditions". For example, at 0.015M sodium ion (no formamide), the melting temperature of perfectly matched long DNA is about 71°C. With a wash at 65°C. (at the same ionic strength), this would allow for approximately a 6% mismatch. To capture more distantly related sequences, those skilled in the art can simply lower the temperature or raise the ionic strength.

[0113]

An appropriate estimate of the melting temperature in 1M NaCl for oligonucleotide probes up to about 20 nucleotides

is given by:

$T_m = (2^{\circ}\text{C per A-T base pair}) + (4^{\circ}\text{C per G-C base pair})$.

Note that the sodium ion concentration in 6× salt sodium citrate (SSC) is 1M (see Suggs et al., Developmental Biology Using
5 Purified Genes, page 683 (Brown and Fox, eds., 1981)).

[0114]

A naturally-occurring nucleic acid encoding a protein such as Pep5, p75, Rho GDI, MAG and p21, may be readily isolated
10 from a cDNA library having PCR primers and hybridization probes containing part of a nucleic acid sequence indicated by, for example, SEQ ID NO. 1, 3, 5, 7, 9, 11, 13, 15, 17 or the like. A preferable nucleic acid encoding Pep5, p75, Rho GDI, MAG and p21 are hybridizable to the whole or part of a sequence
15 as set forth in SEQ ID NO. 1, 3, 5, 7, 9, 11, 13, 15 or 17 under low stringent conditions defined by hybridization buffer essentially containing 1% bovine serum albumin (BSA); 500 mM sodium phosphate (NaPO_4); 1mM EDTA; and 7% SDS at 42°C., and wash buffer essentially containing 2×SSC (600mM NaCl; 60mM
20 sodium citrate); and 0.1% SDS at 50°C, more preferably under low stringent conditions defined by hybridization buffer essentially containing 1% bovine serum albumin (BSA); 500mM sodium phosphate (NaPO_4); 15% formamide; 1mM EDTA; and 7% SDS at 50°C., and wash buffer essentially containing 1×SSC (300mM
25 NaCl; 30mM sodium citrate); and 1% SDS at 50°C., and most preferably under low stringent conditions defined by hybridization buffer essentially containing 1% bovine serum albumin (BSA); 200mM sodium phosphate (NaPO_4); 15% formamide; 1mM EDTA; and 7% SDS at 50°C, and wash buffer essentially
30 containing 0.5×SSC (150mM NaCl; 15 mM sodium citrate); and 0.1% SDS at 65°C.

[0115]

As used herein, the term "probe" refers to a substance for use in searching, which is used in a biological experiment, such as in vitro and/or in vivo screening or the like, including, but not being limited to, for example, a nucleic acid molecule
5 having a specific base sequence or a peptide containing a specific amino acid sequence.

[0116]

Examples of a nucleic acid molecule as a usual probe
10 include one having a nucleic acid sequence having a length of at least 8 contiguous nucleotides, which is homologous or complementary to the nucleic acid sequence of a gene of interest. Such a nucleic acid sequence may be preferably a nucleic acid sequence having a length of at least 9 contiguous nucleotides,
15 more preferably a length of at least 10 contiguous nucleotides, and even more preferably a length of at least 11 contiguous nucleotides, a length of 12 contiguous nucleotides, a length of at least 13 contiguous nucleotides, a length of at least 14 contiguous nucleotides, a length of at least 15 contiguous
20 nucleotides, a length of at least 20 contiguous nucleotides, a length of at least 25 contiguous nucleotides, a length of 30 contiguous nucleotides, a length of at least 40 contiguous nucleotides, or a length of at least 50 contiguous nucleotides. A nucleic acid sequence used as a probe includes a nucleic
25 acid sequence having at least 70% homology to the above-described sequence, more preferably at least 80%, and even more preferably at least 90%, or at least 95%.

[0117]

30 As used herein, the term "search" indicates that a given nucleic acid base sequence is utilized to find other nucleic acid base sequences having a specific function and/or property electronically or biologically, or other methods. Examples

of electronic search include, but are not limited to, BLAST (Altschul et al., J. Mol. Biol. 215:403-410 (1990)), FASTA (Pearson & Lipman, Proc. Natl. Acad. Sci., USA 85:2444-2448 (1988)), Smith and Waterman method (Smith and Waterman, J. Mol. Biol. 147:195-197 (1981)), and Needleman and Wunsch method (Needleman and Wunsch, J. Mol. Biol. 48:443-453 (1970)), and the like. Examples of biological search include, but are not limited to, stringent hybridization, a macroarray in which genomic DNA is attached to a nylon membrane or a microarray (microarray microassay) in which genomic DNA is attached to a glass plate under stringent hybridization, PCR and in situ hybridization, and the like. It is herein intended that Pep5, p75, Rho GDI, MAG and p21 used in the present invention include corresponding genes identified by such an electronic or biological search.

[0118]

As used herein, the percentage of (amino acid, nucleotide, or the like) sequence "identity", "homology" or "similarity" is determined by comparing two optimally aligned sequences over a window of comparison, wherein the portion of a polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i. e. gaps), as compared to the reference sequences (which does not comprise additions or deletions although a gap may occur if the other sequence includes an addition) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residues occur in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the results by 100 to yield the percentage of sequence identity. When used in a search,

homology is evaluated by an appropriate technique selected from various sequence comparison algorithms and programs well known in the art. Examples of such algorithms and programs include, but are not limited to, TBLASTN, BLASTP, FASTA, TFASTA and CLUSTALW (Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA 85(8):2444-2448, Altschul et al., 1990, J. Mol. Biol. 215(3):403-410, Thompson et al., 1994, Nucleic Acids Res. 22(2):4673-4680, Higgins et al., 1996, Methods Enzymol. 266:383-402, Altschul et al., 1990, J. Mol. Biol. 215(3):403-410, Altschul et al., 1993, Nature Genetics 3:266-272). In a particularly preferable embodiment, the homology of a protein or nucleic acid sequence is evaluated using a Basic Local Alignment Search Tool (BLAST) well known in the art (e.g., see Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. USA 87:2267-2268, Altschul et al., 1990, J. Mol. Biol. 215:403-410, Altschul et al., 1993, Nature Genetics 3:266-272, Altschul et al., 1997, Nuc. Acids Res. 25:3389-3402). Particularly, 5 specialized-BLAST programs may be used to perform the following operations to achieve comparison or search:

[0119]

(1) comparison of an amino acid query sequence with a protein sequence database using BLASTP and BLAST3;

(2) comparison of a nucleotide query sequence with a nucleotide sequence database using BLASTN;

(3) comparison of a conceptually translated product in which a nucleotide query sequence (both strands) is converted over 6 reading frames with a protein sequence database using BLASTX;

(4) comparison of all protein query sequences converted over 6 reading frames (both strands) with a nucleotide sequence database using TBLASTN; and

(5) comparison of nucleotide query sequences converted over 6 reading frames with a nucleotide sequence database using TBLASTX.

5 [0120]

The BLAST program identifies homologous sequences by specifying analogous segments called "high score segment pairs" between amino acid query sequences or nucleic acid query sequences and test sequences obtained from preferably a protein sequence database or a nucleic acid sequence database. A large number of the high score segment pairs are preferably identified (aligned) using a scoring matrix which is conventionally well known in the art. Preferably, the scoring matrix is the BLOSUM62 matrix (Gonnet et al., 1992, Science 10 256:1443-1445, Henikoff and Henikoff, 1993, Proteins 15 17:49-61). The PAM or PAM250 matrix may also be used, although they are not as preferable as the BLOSUM62 matrix (e. g., see Schwartz and Dayhoff, eds., 1978, Matrices for Detecting Distance Relationships: Atlas of Protein Sequence and 20 Structure, Washington: National Biomedical Research Foundation). The BLAST program evaluates the statistical significance of all identified high score segment pairs and preferably selects segments which satisfy a threshold level of significance independently defined by a user, such as a 25 user set homology. Preferably, the statistical significance of high score segment pairs is evaluated using Karlin's formula (see Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. USA 87:2267-2268).

30 [0121]

As used herein, the term "primer" refers to a substance required for initiation of a reaction of a macromolecule compound to be synthesized, in a macromolecule synthesis

enzymatic reaction. In a reaction for synthesizing a nucleic acid molecule, a nucleic acid molecule (e. g., DNA, RNA, or the like) which is complementary to part of a macromolecule compound to be synthesized may be used.

5

[0122]

A nucleic acid molecule which is ordinarily used as a primer includes one that has a nucleic acid sequence having a length of at least 8 contiguous nucleotides, which is
10 complementary to the nucleic acid sequence of a gene of interest. Such a nucleic acid sequence preferably has a length of at least 9 contiguous nucleotides, more preferably a length of at least 10 contiguous nucleotides, even more preferably a length of at least 11 contiguous nucleotides, a length of at
15 least 12 contiguous nucleotides, a length of at least 13 contiguous nucleotides, a length of at least 14 contiguous nucleotides, a length of at least 15 contiguous nucleotides, a length of at least 16 contiguous nucleotides, a length of at least 17 contiguous nucleotides, a length of at least 18
20 contiguous nucleotides, a length of at least 19 contiguous nucleotides, a length of at least 20 contiguous nucleotides, a length of at least 25 contiguous nucleotides, a length of at least 30 contiguous nucleotides, a length of at least 40 contiguous nucleotides, and a length of at least 50 contiguous
25 nucleotides. A nucleic acid sequence used as a primer includes a nucleic acid sequence having at least 70% homology to the above-described sequence, more preferably at least 80%, even more preferably at least 90%, and at least 95%. An appropriate sequence as a primer may vary depending on the property of
30 a sequence to be synthesized (amplified). Those skilled in the art can design an appropriate primer depending on a sequence of interest. Such a primer design is well known in the art and may be performed manually or using a computer program (e.g.,

LASERGENE, Primer Select, DNASTar).

[0123]

As used herein, the term "epitope" refers to an antigenic
5 determinant whose structure is clear. Therefore, the term
"epitope" includes a set of amino acid residues which is
involved in recognition by a particular immunoglobulin, or
in the context of T cells, those residues necessary for
recognition by T cell receptor proteins and/or Major
10 Histocompatibility Complex (MHC) receptors. This term is also
used interchangeably with "antigenic determinant" or
"antigenic determinant site". In the field of immunology, in
vivo or in vitro, an epitope is the features of a molecule
(e. g., primary, secondary and tertiary peptide structure,
15 and charge) that form a site recognized by an immunoglobulin,
T cell receptor or HLA molecule. An epitope including a peptide
comprises 3 or more amino acids in a spatial conformation which
is unique to the epitope. Generally, an epitope consists of
at least 5 such amino acids, and more ordinarily, consists
20 of at least 6, 7, 8, 9 or 10 such amino acids. The greater
the length of an epitope, the more the similarity of the epitope
to the original peptide, i. e., longer epitopes are generally
preferable. This is not necessarily the case when the
conformation is taken into account. Methods of determining
25 the spatial conformation of amino acids are known in the art,
and include, for example, X-ray crystallography and
2-dimensional nuclear magnetic resonance spectroscopy.
Furthermore, the identification of epitopes in a given protein
is readily accomplished using techniques well known in the
30 art. See, Geysen et al., Proc. Natl. Acad. Sci. USA (1984)
81: 3998 (general method of rapidly synthesizing peptides to
determine the location of immunogenic epitopes in a given
antigen); U.S. Pat. No. 4,708,871 (procedures for identifying

and chemically synthesizing epitopes of antigens); and Geysen et al., Molecular Immunology (1986) 23: 709 (technique for identifying peptides with high affinity for a given antibody). Antibodies that recognize the same epitope can be identified in a simple immunoassay. Thus, methods for determining an epitopes including a peptide are well known in the art. Such an epitope can be determined using a well-known, common technique by those skilled in the art if the primary nucleic acid or amino acid sequence of the epitope is provided.

[0124]

Therefore, an epitope including a peptide requires a sequence having a length of at least 3 amino acids, preferably at least 4 amino acids, more preferably at least 5 amino acids, at least 6 amino acids, at least 7 amino acids, at least 8 amino acids, at least 9 amino acids, at least 10 amino acids, at least 15 amino acids, at least 20 amino acids, and 25 amino acids. Epitopes may be linear or conformational.

[0125]

(Modification of genes)

In a given protein molecule (e. g., Pep5, p75, Rho GDI, MAG, p21 and the like), a given amino acid contained in a sequence may be substituted with another amino acid in a protein structure, such as a cationic region or a substrate molecule binding site, without a clear reduction or loss of interactive binding ability. A given biological function of a protein is defined by the interactive ability or other property of the protein. Therefore, a particular amino acid substitution may be performed in an amino acid sequence, or at the DNA code sequence level, to produce a protein which maintains the original property after the substitution. Therefore, various modifications of peptides as disclosed herein and DNA encoding

such peptides may be performed without clear losses of biological usefulness.

[0126]

5 When the above-described modifications are designed, the hydrophobicity indices of amino acids may be taken into consideration. The importance of the hydrophobic amino acid indices in providing a protein with an interactive biological function is generally recognized in the art (Kyte. J and
10 Doolittle, R. F., J. Mol. Biol. 157(1):105-132, 1982). The hydrophobic property of an amino acid contributes to the secondary structure of a protein and then regulates interactions between the protein and other molecules (e.g., enzymes, substrates, receptors, DNA, antibodies, antigens,
15 etc.). Each amino acid is given a hydrophobicity index based on the hydrophobicity and charge properties thereof as follows: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7);
20 serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamic acid (-3.5); glutamine (-3.5); aspartic acid (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5)).

25 [0127]

 It is well known that a given amino acid is substituted with another amino acid having a similar hydrophobicity index, and a protein may be produced which still has a biological function similar to that of the original protein (e.g., a
30 protein having an equivalent enzymatic activity). For such an amino acid substitution, the hydrophobicity index is preferably within ± 2 , more preferably within ± 1 , and even more preferably within ± 0.5 . It is understood in the art that such

an amino acid substitution based on the hydrophobicity is efficient. As described in U.S. Pat. No. 4,554,101, amino acid residues are given the following hydrophilicity indices: arginine (+3.0); lysine (+3.0); aspartic acid (+3.0±1);
5 glutamic acid (+3.0±1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5±1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); and tryptophan
10 (-3.4). It is understood that an amino acid may be substituted with another amino acid which has a similar hydrophilicity index and can still provide a biological equivalent. For such an amino acid substitution, the hydrophilicity index is preferably within ±2, more preferably ±1, and even more
15 preferably ±0.5.

[0128]

The term "conservative substitution" as used herein refers to amino acid substitution in which a substituted amino
20 acid and a substituting amino acid have similar hydrophilicity indices or/and hydrophobicity indices. For example, the conservative substitution is carried out between amino acids having a hydrophilicity or hydrophobicity index of within ±2, preferably within ±1, and more preferably within ±0.5. Examples
25 of the conservative substitution include, but are not limited to, substitutions within each of the following residue pairs: arginine and lysine; glutamic acid and aspartic acid; serine and threonine; glutamine and asparagine; and valine, leucine, and isoleucine, which are well known to those skilled in the
30 art.

[0129]

As used herein, the term "variant" refers to a substance,

such as a polypeptide, polynucleotide, or the like, which differs partially from the original substance. Examples of such a variant include a substitution variant, an addition variant, a deletion variant, a truncated variant, an allelic variant, and the like. Examples of such a variant include, but are not limited to, a nucleotide or polypeptide having one or several substitutions, additions and/or deletions or a nucleotide or polypeptide having at least one substitution, addition and/or deletion. The term "allele" as used herein refers to a genetic variant located at a locus identical to a corresponding gene, where the two genes are distinguished from each other. Therefore, the term "allelic variant" as used herein refers to a variant which has an allelic relationship with a given gene. Such an allelic variant ordinarily has a sequence the same as or highly similar to that of the corresponding allele, and ordinarily has almost the same biological activity, though it rarely has different biological activity. The term "species homolog" or "homolog" as used herein refers to one that has an amino acid or nucleotide homology with a given gene in a given species (preferably at least 60% homology, more preferably at least 80%, at least 85%, at least 90%, and at least 95% homology). A method for obtaining such a species homolog is clearly understood from the description of the present specification. The term "orthologs" is also called orthologous genes and refers to genes in different species derived from speciation from a common ancestry. For example, in the case of the hemoglobin gene family having multigene structure, human and mouse α -hemoglobin genes are orthologs, while the human α -hemoglobin gene and the human β -hemoglobin gene are paralogs (genes arising from gene duplication). Orthologs are useful for estimation of molecular phylogenetic trees. Usually, orthologs in different species may have a function similar

to that of the original species. Therefore, orthologs of the present invention may be useful in the present invention.

[0130]

5 As used herein, the term "conservative (or conservatively modified) variant" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refer to those nucleic acids which encode identical or essentially
10 identical amino acid sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For example, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon,
15 the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations" which represent one species of conservatively modified variation. Every nucleic acid sequence herein which encodes a polypeptide
20 also describes every possible silent variation of the nucleic acid. Those skilled in the art will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally
25 identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence. Preferably, such modification may be performed while avoiding substitution of cysteine which is an amino acid capable of largely affecting the higher-order
30 structure of a polypeptide. Examples of a method for such modification of a base sequence include cleavage using a restriction enzyme or the like; ligation or the like by treatment using DNA polymerase, Klenow fragments, DNA ligase,

or the like; and a site specific base substitution method using synthesized oligonucleotides (specific-site directed mutagenesis; Mark Zoller and Michael Smith, Methods in Enzymology, 100, 468-500(1983)). Modification can be
5 performed using methods ordinarily used in the field of molecular biology.

[0131]

10 In order to prepare functionally equivalent polypeptides, amino acid additions, deletions, or modifications can be performed in addition to amino acid substitutions. Amino acid substitution(s) refers to the replacement of at least one amino acid of an original peptide with different amino acids, such as the replacement of 1 to 10 amino acids, preferably
15 1 to 5 amino acids, and more preferably 1 to 3 amino acids with different amino acids. Amino acid addition(s) refers to the addition of at least one amino acid to an original peptide chain, such as the addition of 1 to 10 amino acids, preferably 1 to 5 amino acids, and more preferably 1 to 3 amino acids
20 to an original peptide chain. Amino acid deletion(s) refers to the deletion of at least one amino acid, such as the deletion of 1 to 10 amino acids, preferably 1 to 5 amino acids, and more preferably 1 to 3 amino acids. Amino acid modification includes, but is not limited to, amidation, carboxylation,
25 sulfation, halogenation, alkylation, glycosylation, phosphorylation, hydroxylation, acylation (e.g., acetylation), and the like. Amino acids to be substituted or added may be naturally-occurring or nonnaturally-occurring amino acids, or amino acid analogs. Naturally-occurring amino acids
30 are preferable.

[0132]

As used herein, the term "peptide analog" or "peptide

derivative" refers to a compound which is different from a peptide but has at least one chemical or biological function equivalent to the peptide. Therefore, a peptide analog includes one that has at least one amino acid analog or amino acid derivative addition or substitution with respect to the original peptide. A peptide analog has the above-described addition or substitution so that the function thereof is substantially the same as the function of the original peptide (e. g., a similar pKa value, a similar functional group, a similar binding manner to other molecules, a similar water-solubility, and the like). Such a peptide analog can be prepared using a technique well known in the art. Therefore, a peptide analog may be a polymer containing an amino acid analog.

[0133]

Similarly, the term "polynucleotide analog" or "nucleic acid analog" refers to a compound which is different from a polynucleotide or a nucleic acid but has at least one chemical function or biological function equivalent to that of a polynucleotide or a nucleic acid. Therefore, a polynucleotide analog or a nucleic acid analog includes one that has at least one nucleotide analog or nucleotide derivative addition or substitution with respect to the original peptide.

[0134]

Nucleic acid molecules as used herein includes one in which a part of the sequence of the nucleic acid is deleted or is substituted with other base(s), or an additional nucleic acid sequence is inserted, as long as a polypeptide expressed by the nucleic acid has substantially the same activity as that of the naturally-occurring polypeptide, as described

above. Alternatively, an additional nucleic acid may be linked to the 5' terminus and/or 3' terminus of the nucleic acid. The nucleic acid molecule may include one that is hybridizable to a gene encoding a polypeptide under stringent conditions and encodes a polypeptide having substantially the same function as that of that polypeptide. Such a gene is known in the art and can be used in the present invention.

[0135]

The above-described nucleic acid can be obtained by a well-known PCR method, and also can be obtained by chemical synthesis. This method may be combined with, for example, site-specific mutagenesis, hybridization, or the like.

[0136]

As used herein, the term "substitution, addition or deletion" for a polypeptide or a polynucleotide refers to the substitution, addition or deletion of an amino acid or its substitute, or a nucleotide or its substitute with respect to the original polypeptide or polynucleotide. Such substitution, addition or deletion is well known in the art, and examples of such techniques include a site-specific mutagenesis technique and the like. A polypeptide or a polynucleotide may have any number of substitutions, additions, or deletions as long as the number is equal to or greater than 1. The number can be as large as a variant having such a number of substitutions, additions or deletions maintains an intended function (e.g., the information transfer function of hormones and cytokines, and the like). For example, such a number may be one or several, and preferably within 20% or 10% of the full length, or no more than 100, no more than 50, no more than 25, or the like.

[0137]

(General Techniques)

Molecular biological techniques, biochemical techniques, and microorganism techniques as used herein are well known in the art and commonly used, and are described in, for example, Sambrook J. et al. (1989), *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor and its 3rd Ed. (2001); Ausubel, F. M. (1987), *Current Protocols in Molecular Biology*, Greene Pub. Associates and Wiley-Interscience; Ausubel, F. M. (1989), *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Greene Pub. Associat ES and Wiley-Interscience; Sambrook, J. et al. (1989). *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor and 3rd Ed. (2001); Innis, M. A. (1990), *PCR Protocols: A Guide to Methods and Applications*, Academic Press; Ausubel, F. M. (1992), *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Greene Pub. Associates; Ausubel, F. M. (1995), *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Greene Pub. Associates; Innis, M. A. et al. (1995), *PCR Strategies*, Academic Press; Ausubel, F. M. (1999), *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Wiley, and annual updates; Sninsky, J. J. et al. (1999), *PCR Applications: Protocols for Functional Genomics*, Academic Press; Special issue, Jikken Igaku [Experimental Medicine] "Experimental Method for Gene Introduction & Expression Analysis", Yodo-sha, 1997; and the like. Relevant portions (or possibly the entirety) of each of these publication are herein incorporated by reference.

[0138]

DNA synthesis techniques and nucleic acid chemistry

for preparing artificially synthesized genes are described in, for example, Gait, M. J. (1985), *Oligonucleotide Synthesis: A Practical Approach*, IRL Press; Gait, M. J. (1990), *Oligonucleotide Synthesis: A Practical Approach*, IRL Press; 5 Eckstein, F. (1991), *Oligonucleotides and Analogues: A Practical Approach*, IRL Press; Adams, R. L. et al. (1992), *The Biochemistry of the Nucleic Acids*, Chapman & Hall; Shabarova, Z. et al. (1994), *Advanced Organic Chemistry of Nucleic Acids*, Weinheim; Blackburn, G. M. et al. (1996), *Nucleic Acids in 10 Chemistry and Biology*, Oxford University Press; Hermanson, G. T. (1996), *Bioconjugate Techniques*, Academic Press; and the like. Relevant portions of these publications are herein incorporated by reference.

15 [0139]

(Genetic Engineering)

Pep5, p75, Rho GDI, MAG and p21, and fragments and variants thereof as used herein can be produced by genetic engineering techniques.

20

[0140]

When a gene is mentioned herein, the term "vector" or "recombinant vector" refers to a vector capable of transferring a polynucleotide sequence of interest to a target cell. Such 25 a vector is capable of self-replication or incorporation into a chromosome in a host cell such as a prokaryotic cell, yeast, an animal cell, a plant cell, an insect cell, an individual animal, and an individual plant, and contains a promoter at a site suitable for transcription of a polynucleotide of the 30 present invention. Among vectors, a vector suitable for cloning is referred to as "cloning vector". Such a cloning vector ordinarily contains a multiple cloning site containing a plurality of restriction sites. Restriction sites and multiple

cloning sites are well known in the art and may be appropriately or optionally used depending on the purpose. The technology is described in references as described herein (e.g., Sambrook et al. (supra)).

5

[0141]

As used herein, the term "expression vector" refers to a nucleic acid sequence comprising a structural gene and a promoter for regulating expression thereof, and in addition, various regulatory elements in a state that allows them to operate within host cells. The regulatory element may include, preferably, terminators, selectable markers such as drug-resistance genes, and enhancers. It is well known to those skilled in the art that the type of an organism (e. g., an animal) expression vector and the type of a regulatory element may vary depending on the host cell.

[0142]

As used herein, a "recombinant vector" for prokaryotic cells includes, for example, pcDNA 3(+), pBluescript-SK(+/-), pGEM-T, pEF-BOS, pEGFP, pHAT, pUC18, pFT-DEST^T, ^M42GATEWAY (Invitrogen), and the like.

[0143]

As used herein, a "recombinant vector" for animal cells includes, for example, pcDNA I/Amp, pcDNA I, pCDM8 (all commercially available from Funakoshi), pAGE107 [Japanese Laid-Open Publication No. 3-229 (Invitrogen)], pAGE103 [J. Biochem., 101, 1307 (1987)], pAMo, pAMoA [J. Biol. Chem., 268, 22782-22787 (1993)], retroviral expression vectors based on Murine Stem Cell Virus (MSCV), pEF-BOS, pEGFP, and the like.

[0144]

As used herein, the term "terminator" refers to a sequence which is located downstream of a protein-encoding region of a gene and which is involved in the termination of transcription when DNA is transcribed into mRNA, and the
5 addition of a poly A sequence. It is known that a terminator contributes to the stability of mRNA, and has an influence on the amount of gene expression.

[0145]

10 As used herein, the term "promoter" refers to a base sequence which determines the initiation site of transcription of a gene and is a DNA region which directly regulates the frequency of transcription. Transcription is started by RNA polymerase binding to a promoter. Therefore, a portion of a
15 given gene which functions as a promoter is herein referred to as a "promoter portion". A promoter region is usually located within about 2 kbp upstream of the first exon of a putative protein coding region. Therefore, it is possible to estimate a promoter region by predicting a protein coding region in
20 a genomic base sequence using DNA analysis software. A putative promoter region is usually located upstream of a structural gene, but depending on the structural gene, i.e., a putative promoter region may be located downstream of a structural gene. Preferably, a putative promoter region is located within about
25 2 kbp upstream of the translation initiation site of the first exon.

[0146]

30 As used herein, the term "enhancer" refers to a sequence which is used so as to enhance the expression efficiency of a gene of interest. Such an enhancer is well known in the art. One or more enhancers may be used, or no enhancer may be used.

[0147]

As used herein, the term "operatively linked" indicates that a desired sequence is located such that expression (operation) thereof is under control of a transcription and translation regulatory sequence (e. g., a promoter, an enhancer, and the like) or a translation regulatory sequence. In order for a promoter to be operatively linked to a gene, typically, the promoter is located immediately upstream of the gene, but a promoter is not necessarily adjacent to a structural gene.

[0148]

Any technique may be used herein for introduction of a nucleic acid molecule into cells, including, for example, transformation, transduction, transfection, and the like. Such a nucleic acid molecule introduction technique is well known in the art and commonly used, and is described in, for example, Ausubel F. A. et al., editors, (1988), Current Protocols in Molecular Biology, Wiley, New York, N.Y.; Sambrook J. et al. (1987) Molecular Cloning: A Laboratory Manual, 2nd Ed. and its 3rd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Special issue, Jikken Igaku [Experimental Medicine] "Experimental Method for Gene Introduction & Expression Analysis", Yodo-sha, 1997; and the like. Gene introduction can be confirmed by method as described herein, such as Northern blotting analysis and Western blotting analysis, or other well-known, common techniques.

[0149]

Any of the above-described methods for introducing DNA into cells can be used as an vector introduction method, including, for example, transfection, transduction, transformation, and the like (e.g., a calcium phosphate method,

a liposome method, a DEAE dextran method, an electroporation method, a particle gun (gene gun) method, and the like).

[0150]

5 As used herein, the term "transformant" refers to the whole or a part of an organism, such as a cell, which is produced by transformation. Examples of a transformant include a prokaryotic cell, yeast, an animal cell, a plant cell, an insect cell, and the like. Transformants may be referred to as
10 transformed cells, transformed tissue, transformed hosts, or the like, depending on the subject. A cell used herein may be a transformant.

[0151]

15 When a prokaryotic cell is used herein for genetic operations or the like, the prokaryotic cell may be of, for example, genus *Escherichia*, genus *Serratia*, genus *Bacillus*, genus *Brevibacterium*, genus *Corynebacterium*, genus *Microbacterium*, genus *Pseudomonas*, or the like. Specifically,
20 the prokaryotic cell is, for example, *Escherichia coli* XL1-Blue, *Escherichia coli* XL2-Blue, *Escherichia coli* DH1, or the like.

[0152]

25 Examples of an animal cell as used herein include a mouse myeloma cell, a rat myeloma cell, a mouse hybridoma cell, a Chinese hamster ovary (CHO) cell, a baby hamster kidney (BHK) cell, an African green monkey kidney cell, a human leukemic cell, HBT5637 (Japanese Laid-Open Publication No. 63-299), a human colon cancer cell line, and the like. The mouse myeloma
30 cell includes ps20, NSO, and the like. The rat myeloma cell includes YB2/0 and the like. A human embryo kidney cell includes HEK293 (ATCC:CRL-1573) and the like. The human leukemic cell includes BALL-1 and the like. The African green monkey kidney

cell includes COS-1, COS-7, and the like. The human colon cancer cell line includes HCT-15, and the like. A human neuroblastoma includes SK-N-SH, SK-N-SH-5Y, and the like. A mouse neuroblastoma includes Neuro2A, and the like.

5

[0153]

Any method for introduction of DNA can be used herein as a method for introduction of a recombinant vector, including, for example, a calcium chloride method, an electroporation method [Methods. Enzymol., 194, 182 (1990)], a lipofection method, spheroplast method [Proc. Natl. Acad. Sci. USA, 84, 1929 (1978)], a lithium acetate method [J. Bacteriol., 153, 163 (1983)], a method described in Proc. Natl. Acad. Sci. USA, 75, 1929 (1978), and the like.

15

[0154]

A retrovirus infection method as used herein is well known in the art as described in, for example, Current Protocols in Molecular Biology (supra) (particularly, Units 9.9-9.14), and the like. Specifically, for example, embryonic stem cells are trypsinized into a single-cell suspension, followed by co-culture with the culture supernatant of virus-producing cells (packaging cell lines) for 1-2 hours, thereby obtaining a sufficient amount of infected cells.

25

[0155]

The transient expression of Cre enzyme, DNA mapping on a chromosome, and the like, which are used herein in a method for removing a genome, a gene locus, or the like, are well known in the art, as described in Kenichi Matsubara and Hiroshi Yoshikawa, editors, Saibo-Kogaku [Cell Engineering], special issue, "Experiment Protocol Series "FISH Experiment Protocol from Human Genome Analysis to Chromosome/Gene diagnosis",

30

Shujun-sha (Tokyo), and the like.

[0156]

Gene expression (e. g., mRNA expression, polypeptide
5 expression) may be "detected" or "quantified" by an appropriate
method, including mRNA measurement and immunological
measurement method. Examples of the molecular biological
measurement method include a Northern blotting method, a dot
10 blotting method, a PCR method, and the like. Examples of the
immunological measurement method include an ELISA method, an
RIA method, a fluorescent antibody method, a Western blotting
method, an immunohistological staining method, and the like,
where a microtiter plate may be used. Examples of a
quantification method include an ELISA method, an RIA method,
15 and the like. A gene analysis method using an array (e.g.,
a DNA array and a protein array) may be used. The DNA array
is widely reviewed in Saibo-Kogaku [Cell Engineering], special
issue, "DNA Microarray and Up-to-date PCR Method", edited by
Shujun-sha. The protein array is described in detail in Nat
20 Genet. 2002 Dec; 32 Suppl:526-32. Examples of a method for
analyzing gene expression include, but are not limited to,
an RT-PCR method, a RACE method, an SSCP method, an im-
munoprecipitation method, a two-hybrid system, an in vitro
translation method, and the like in addition to the
25 above-described techniques. Such further analysis methods are
described in, for example, "Genome Analysis Experimental
Method, Yusuke Nakamura's Labo-Manual, edited by Yusuke
Nakamura, Yodo-sha (2002), and the like. All of the
above-described publications are herein incorporated by
30 reference.

[0157]

As used herein, the term "amount of expression" refers

to the amount of a polypeptide or mRNA expressed in a subject cell. The amount of expression includes the amount of expression at the protein level of a polypeptide of the present invention evaluated by any appropriate method using an antibody
5 of the present invention, including immunological measurement methods such as an ELISA method, an RIA method, a fluorescent antibody method, a Western blotting method, an immunohistological staining method, and the like, or the amount of expression at the mRNA level of a polypeptide of the present
10 invention evaluated by any appropriate method, including molecular biological measurement methods such as a Northern blotting method, a dot blotting method, a PCR method, and the like. The term "change in the amount of expression" indicates that an increase or decrease in the amount of expression at
15 the protein or mRNA level of a polypeptide of the present invention evaluated by an appropriate method including the above-described immunological measurement method or molecular biological measurement method.

20 [0158]

As used herein, the term "upstream" refers to the position closer to the 5' terminus than a specific reference point.

25 [0159]

As used herein, the term "downstream" refers to the position closer to the 3' terminus than a specific reference point.

30 [0160]

As used herein, the term "base paired" and "Watson & Crick base paired" have the same meaning and refer to nucleotides which can be bound together by hydrogen bonds based

on the sequence identity that an adenine residue is bound to a thymine residue or a uracil residue via two hydrogen bonds and a cytosine residue is bound to a guanine residue via three hydrogen bonds, as seen in double-stranded DNA (see Stryer, 5 L., Biochemistry, 4th edition, 1995).

[0161]

As used herein, the term "complementary" or "complement" refers to a polynucleotide sequence such that the 10 whole complementary region thereof is capable of Watson-Crick base pairing with another specific polynucleotide. In the present invention, when each base of a first polynucleotide pairs with a corresponding complementary base, the first polynucleotide is regarded as being complementary to a second 15 polynucleotide. Complementary bases are generally A and T (or A and U) or C and G. As used herein, the term "complement" is used as a synonym for the terms "complementary polynucleotide", "complementary nucleic acid" and "complementary nucleotide sequence". These terms are applied to 20 a pair of polynucleotides based on the sequence, but not a specific set of two polynucleotides which are virtually bound together.

[0162]

25 (Polypeptide Production Method)

A transformant derived from a microorganism, an animal cell, or the like, which possesses a recombinant vector into which DNA encoding a polypeptide of the present invention (e. g., Pep5, p75, Rho GDI, MAG, p21, and the like) is 30 incorporated, is cultured according to an ordinary culture method. The polypeptide of the present invention is produced and accumulated. The polypeptide of the present invention is collected from the culture, thereby making it possible to

produce the polypeptide of the present invention.

[0163]

5 The transformant of the present invention can be
cultured on a culture medium according to an ordinary method
for use in culturing host cells. A culture medium for a
transformant obtained from a prokaryote such as *E. coli* or
a eukaryote such as yeast as a host may be either a
naturally-occurring culture medium or a synthetic culture
10 medium as long as the medium contains a carbon source, a nitrogen
source, inorganic salts, and the like which an organism of
the present invention can assimilate and the medium allows
efficient culture of the transformant.

15 [0164]

The carbon source includes any one that can be
assimilated by the organism. Carbohydrates such as glucose,
fructose, sucrose, molasses containing these, starch, starch
hydrolysate and the like, organic acids such as acetic acid,
20 propionic acid and the like, and alcohols such as ethanol,
propanol and the like can be used.

[0165]

25 As the nitrogen source includes ammonium salts of
inorganic or organic acids such as ammonia, ammonium chloride,
ammonium sulfate, ammonium acetate, ammonium phosphate, and
the like, other nitrogen-containing substances, and peptone,
meat extract, yeast extract, corn steep liquor, casein
hydrolysate, soybean cake, and soybean cake hydrolysate,
30 various fermentation bacteria and digestion products thereof
and the like can be used.

[0166]

As salts of inorganic acids, such as potassium (I) phosphate, potassium (II) phosphate, magnesium phosphate, magnesium phosphate, sodium chloride, iron (I) sulfate, manganese sulfate, copper sulfate, calcium carbonate, and the
5 like, can be used. Culture is performed under aerobic conditions for shaking culture, deep aeration agitation culture, or the like.

[0167]

10 Culture temperature is preferably 15 to 40°C, and culture time is ordinarily 5 hours to 7 days. The pH of culture medium is maintained at 3.0 to 9.0. The adjustment of pH is carried out using inorganic or organic acid, alkali solution, urea, calcium carbonate, ammonia, or the like. An antibiotic,
15 such as ampicillin, tetracycline, or the like, may be added to culture medium during cultivation, if necessary.

[0168]

When culturing a microorganism which has been
20 transformed using an expression vector where an inducible promoter is used as a promoter, an inducer may be added to the culture medium. For example, when a microorganism, which has been transformed using an expression vector where a lac promoter is used as a promoter, is cultured, isopropyl- β -D-thiogalactopyranoside or the like may be added to the
25 culture medium. When a microorganism, which has been transformed using an expression vector where a trp promoter is used as a promoter, is cultured, indole acrylic acid or the like may be added to culture medium. A cell or an organ
30 into which a gene has been introduced can be cultured in a large volume using a jar fermenter.

[0169]

For example, when an animal cell is used, a culture medium of the present invention for culturing the cell includes a commonly used RPMI1640 culture medium (The Journal of the American Medical Association, 199, 519 (1967)), Eagle's MEM
5 culture medium (Science, 122, 501 (1952)), DMEM culture medium (Virology, 8, 396 (1959)), 199 culture medium (Proceedings of the Society for the Biological Medicine, 73, 1 (1950)) or these culture media supplemented with fetal bovine serum or the like.

10

[0170]

Culture is normally carried out for 1 to 7 days under conditions such as pH 6 to 8, 25 to 40°C., 5% CO₂. An antibiotic, such as kanamycin, penicillin, streptomycin, or the like may
15 be added to culture medium during cultivation, if necessary.

[0171]

A polypeptide of the present invention can be isolated or purified from a culture of a transformant, which has been
20 transformed with a nucleic acid sequence encoding the polypeptide of the present invention, using an ordinary method for isolating or purifying enzymes, which are well known and commonly used in the art. For example, when a polypeptide of the present invention is secreted outside of a cell of the
25 transformant for producing the polypeptide, the culture is processed by a method such as centrifugation or the like to obtain a soluble fraction. A purified specimen can be obtained from the soluble fraction by a technique, such as solvent extraction, salting-out/desalting with ammonium sulfate or
30 the like, precipitation with organic solvent, anion exchange chromatography with a resin such as diethylaminoethyl (DEAE)-Sephadex, DIAION HPA-75 (Mitsubishi Kasei Corporation), cation exchange chromatography with a resin such as

S-Sepharose FF (Pharmacia), hydrophobic chromatography with a resin such as buthylsepharose and phenylsepharose, gel filtration with a molecular sieve, affinity chromatography, chromatofocusing, electrophoresis such as isoelectric focusing electrophoresis, and the like.

[0172]

When a polypeptide (e.g., Pep5, p75, Rho GDI, MAG, p21, and the like) of the present invention is accumulated in a dissolved form within a transformant cell for producing the polypeptide, the culture is subjected to centrifugation to collect cells in the culture. The cells are washed, followed by pulverization of the cells using a ultrasonic pulverizer, a French press, MANTON GAULIN homogenizer, Dinomil, or the like, to obtain a cell-free extract solution. A purified specimen can be obtained from a supernatant obtained by centrifuging the cell-free extract solution by a technique, such as solvent extraction, salting-out/desalting with ammonium sulfate or the like, precipitation with organic solvent, anion exchange chromatography with a resin such as diethylaminoethyl (DEAE)-Sepharose and DIAION HPA-75 (Mitsubishi Kasei Corporation), cation exchange chromatography with a resin such as S-Sepharose FF (Pharmacia), hydrophobic chromatography with a resin such as buthylsepharose and phenylsepharose, gel filtration with a molecular sieve, affinity chromatography, chromatofocusing, electrophoresis such as isoelectric focusing electrophoresis, and the like.

[0173]

When the polypeptide of the present invention has been expressed and formed insoluble bodies within cells, the cells are harvested, pulverized, and centrifuged. From the resulting

precipitate fraction, the polypeptide of the present invention is collected using a commonly used method. The insoluble polypeptide is solubilized using a polypeptide denaturant. The resulting solubilized solution is diluted or dialyzed into
5 a denaturant-free solution or a dilute solution, where the concentration of the polypeptide denaturant is too low to denature the polypeptide. The polypeptide of the present invention is allowed to form a normal three-dimensional structure, and the purified specimen is obtained by isolation
10 and purification as described above.

[0174]

Purification can be carried out in accordance with a commonly used protein purification method [J. Evan. Sadler
15 et al.: Methods in Enzymology, 83, 458]. Alternatively, the polypeptide of the present invention can be produced as a fusion protein with other proteins, and the fusion protein can be purified using affinity chromatography using a substance having affinity to the fusion protein [(Akio Yamakawa,
20 Experimental Medicine, 13, 469-474 (1995)]. For example, in accordance with a method of Lowe et al., [Proc. Natl. Acad. Sci., USA, 86, 8227-8231 (1989), Genes Develop., 4, 1288 (1990)], a polypeptide of the present invention can be produced as a fusion protein with protein A followed by purification with
25 affinity chromatography using immunoglobulin G.

[0175]

A fusion protein of the polypeptide of the present invention with a FLAG peptide is produced, followed by
30 purification with affinity chromatography using anti-FLAG antibodies [Proc. Natl. Acad. Sci., USA, 86, 8227 (1989), Genes Develop., 4, 1288 (1990)].

[0176]

The polypeptide of the present invention can be purified with affinity chromatography using antibodies to the polypeptide. The polypeptide of the present invention can be produced using an *in vitro* transcription/translation system in accordance with a known method [J. Biomolecular NMR, 6, 129-134; Science, 242, 1162-1164; J. Biochem., 110, 166-168 (1991)].

10 [0177]

The polypeptide of the present invention can also be produced by a chemical synthesis method, such as the Fmoc method (fluorenylmethyloxycarbonyl method), the tBoc method (t-butyloxycarbonyl method), or the like, based on the amino acid information thereof. The peptide can be chemically synthesized using a peptide synthesizer (manufactured by Advanced ChemTech, Applied Biosystems, Pharmacia Biotech, Protein Technology Instrument, Synthecell-Vega, PerSeptive, Shimazu, or the like).

20

[0178]

The structure of the purified polypeptide of the present invention can be carried out by methods commonly used in protein chemistry (see, for example, Hisashi Hirano. "Protein Structure Analysis for Gene Cloning", published by Tokyo Kagaku Dojin, 1993). The physiological activity of a novel ps20-like polypeptide of the present invention can be measured in accordance with a known measurement method [Cell, 75, 1389(1993), J. Cell Bio. 1146, 233 (1999), Cancer Res. 58, 1238 (1998), Neuron 17, 1157 (1996), Science 289, 1197 (2000)].

30

[0179]

(Method for Producing Mutant Polypeptide)

Amino acid deletion, substitution or addition (including fusion) of the polypeptide of the present invention (e.g., Pep5, p75, Rho GDI, MAG, p21, and the like) can be carried out by a site-specific mutagenesis method which is a well known technique. One or several amino acid deletions, substitutions or additions can be carried out in accordance with methods described in Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press (1989); Current Protocols in Molecular Biology, Supplement 1-38, John Wiley & Sons (1987-1997); Nucleic Acids Research, 10, 6487 (1982); Proc. Natl. Acad. Sci., USA, 79, 6409 (1982); Gene, 34, 315 (1985); Nucleic Acids Research, 13, 4431 (1985); Proc. Natl. Acad. Sci USA, 82, 488 (1985); Proc. Natl. Acad. Sci., USA, 81, 5662 (1984); Science, 224, 1431 (1984); PCT WO85/00817(1985); Nature, 316, 601 (1985); and the like.

[0180]

(Immunochemistry)

Preparation of antibodies which recognize the polypeptide of the present invention (e.g., Pep5, p75, Rho GDI, MAG, p21, and the like) are also well known in the art. For example, preparation of polyclonal antibodies can be carried out by administering a purified specimen of the whole or a partial fragment of an obtained polypeptide or a peptide having a part of the amino acid sequence of the protein of the present invention, as an antigen, to an animal.

[0181]

To produce antibodies, a rabbit, a goat, a rat, a mouse, a hamster, or the like can be used as an animal to which an antigen is administered. The dose of the antigen is preferably 50 to 100 μ g per animal. When a peptide is used as an antigen, the peptide is preferably coupled via covalent bond to a carrier

protein, such as keyhole limpet hemocyanin, bovine thyroglobulin, or the like. A peptide used as an antigen can be synthesized using a peptide synthesizer. The antigen is administered every 1 to 2 weeks after a first administration
5 a total 3 to 10 times. 3 to 7 days after each administration, blood is collected from the venous plexus of eye grounds, and whether or not the serum reacts with the antigen which has been used for immunization is determined by an enzyme immunoassay [Enzyme Immunoassay (ELISA): published by
10 Igaku-syoin 1976; Antibodies-A Laboratory Manual, Cold Spring Harbor Laboratory (1988)]; and the like.

[0182]

Serum is obtained from a non-human mammal whose serum
15 exhibits a sufficient antibody titer to an antigen. From the serum, polyclonal antibodies can be isolated and purified using well known techniques. Production of monoclonal antibodies is also well known in the art. In order to prepare antibody producing cells, a rat whose serum exhibits a sufficient
20 antibody titer for fragments of a polypeptide of the present invention which has been used for immunization, is used as a source for antibody producing cells, which are fused with myeloma cells to prepare hybridomas. Thereafter, a hybridoma specifically reacting with the fragments of the polypeptide
25 of the present invention is selected using enzyme immunoassays. A monoclonal antibody produced by the thus-obtained hybridoma can be used for various purposes.

[0183]

30 Such an antibody can be used for an immunological method of detecting the polypeptide of the present invention, for example. Examples of an immunological method of detecting the polypeptide of the present invention using the antibody of

the present invention include an ELISA method using microtiter plates, a fluorescent antibody method, a Western blotting method, an immunohistological method, and the like.

5 [0184]

Further, the antibody of the present invention can be used for immunological methods for quantifying the polypeptide of the present invention polypeptide. Examples of the method for quantifying the polypeptide of the present invention
10 include a sandwich ELISA method using two monoclonal antibodies for different two epitopes of the polypeptide of the present invention, among those which react with the polypeptide of the present invention; a radioimmunoassay using the protein of the present invention labeled with a radioactive isotope,
15 such as ^{126}I or the like, and the like.

[0185]

Methods for quantifying mRNA for the polypeptide of the present invention polypeptide are well known in the art.
20 For example, the above-described oligonucleotides prepared from the polynucleotide or DNA of the present invention can be used to quantify the amount of expression of DNA encoding the polypeptide of the present invention based on the mRNA level using Northern hybridization method or PCR method. Such
25 a technique is well known in the art and is described in reference described herein.

[0186]

These polynucleotides may be obtained, and the
30 nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of an antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides

(e. g., as described in Kutmeier et al., BioTechniques 17: 242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligation of those
5 oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0187]

A polynucleotide encoding an antibody can be produced
10 from a nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but when the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized, or obtained from a suitable source (e. g., an
15 antibody cDNA library, or a cDNA library generated from any tissue or cells expressing the antibody (e. g., hybridoma cells selected to express an antibody of the present invention), or nucleic acids (preferably poly A+RNA) isolated therefrom) by PCR amplification using synthetic primers hybridizable to
20 the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, for example, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids produced by PCR may be cloned into replicable cloning vectors
25 using any method well known in the art.

[0188]

Once the nucleotide sequence and corresponding amino acid sequence of an antibody is determined, the nucleotide
30 sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences (e. g., recombinant DNA techniques, site directed mutagenesis, PCR, and the like (see, for example, the techniques described

in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both
5 incorporated by reference herein in their entireties), to produce antibodies having a different amino acid sequence, for example, to create amino acid substitutions, deletions, and/or insertions.

10 [0189]

In a specific embodiment, the amino acid sequence of heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art (e. g.,
15 by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability). Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions (e. g., into human framework regions to
20 humanize a non-human antibody) as described above. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e. g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the
25 polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the present invention. Preferably, as discussed above, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino
30 acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide

bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the technique of the art.

5

[0190]

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608
10 (1984); Takeda et al., Nature 314: 452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described above, a chimeric antibody is a molecule in which different
15 portions are derived from different animal species. Such a molecule has a variable region derived from a murine mAb and a human immunoglobulin constant region (e. g., humanized antibodies).

20 [0191]

Known techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778; Bird, Science 242:423-42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989))
25 can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra
30 et al., Science 242:1038-1041 (1988)).

[0192]

(Methods of Producing Antibodies)

The antibodies of the present invention can be produced by any method known in the art for the synthesis of antibodies, by chemical synthesis, or preferably, by recombinant expression techniques.

5

[0193]

Recombinant expression of an antibody of the present invention, or fragment, derivative or analog thereof (e. g., a heavy or light chain of an antibody of the present invention) requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the present invention has been obtained, a vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art may be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in viva genetic recombination. The present invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the present invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e. g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Pat. No. 5,122,464) and

the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

[0194]

5 The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the present invention. Thus, the present invention includes host cells containing a polynucleotide encoding an antibody
10 of the present invention, or a heavy or light chain thereof, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire
15 immunoglobulin molecule, as detailed below.

[0195]

 In embodiments related to the present invention, pharmaceutical compositions (e. g., vaccine compositions)
20 may be provided for prophylactic or therapeutic applications. Such compositions generally include immunogenic polypeptides or polynucleotides and immune stimulating agents (e. g., adjuvants) of the present invention.

25 [0196]

(Screening)

 As used herein, the term "screening" refers to selection of a target, such as an organism, a substance, or the like having a specific property of interest from a population
30 containing a number of elements using a specific operation/evaluation method. For screening, an agent (e. g., an antibody), a polypeptide or a nucleic acid molecule of the present invention can be used. Screening may be performed using

a system of *in vitro*, *in vivo*, or the like using a real substance or alternatively a library generated *in silico* (with a system using a computer). It will be understood that the present invention encompasses compounds having desired activity
5 obtained by screening. The present invention is also intended to provide drugs which are produced by computer modeling based on the disclosures of the present invention.

[0197]

10 (Nervous Diseases and Nerve Regeneration)

The term "nervous disease" or "neurological disease" are used herein interchangeably to refer to the discontinuation, termination or disorder of a function, a structure, an organ, or the like of a nerve. The term typically refers to a lesion
15 satisfying at least two of the following criteria: 1) the presence of a pathogenic substance; 2) the presence of a symptom and/or a syndrome capable of being clearly indicated; and 3) a corresponding anatomical change. Examples of nervous diseases include, but are not limited to, cerebrovascular
20 disorders (e. g., cerebral hemorrhage, subarachnoid hemorrhage, cerebral infarction, transient (cerebral) ischemic attack (TIA), cerebral arteriosclerosis, Binswanger disease, cerebral sinus thrombosis/cerebral phlebothrombosis, hypertensive encephalopathy, temporal arteritis, transient
25 global amnesia (TGA), moya-moya disease, fibromuscular hyperplasia internal carotid artery/cavernous sinus/fistula, chronic subdural hematoma, amyloid angiopathy (see Alzheimer disease), and the like); circulatory disorder of the spinal
30 cords (e. g., spinal infarct, transient spinal ischemia, spinal hemorrhage, circulatory deformity of the spinal cord, spinal subarachnoid hemorrhage, subacute necrotizing myelitis, and the like); infective and inflamational disorders (e. g., meningitis, encephalitis, Herpes simplex encephalitis,

Japanese encephalitis, other encephalitises, rabies, slow virus disease (e. g., subacute sclerosing panencephalitis, progressive multifocal leukoencephalitis, Creutzfeldt-Jakob disease, and the like), neural Behcet disease, chorea minor AIDS dementia syndrome, neuro syphilis, cerebral abscess, spinal epidural abscess, HTLV-I-associated myelopathy, poliomyelitis); demyelinating diseases (multiple sclerosis, acute disseminated encephalomyelitis, Baló's concentric sclerosis, inflammatory universal sclerosis, leukodystrophy, metachromatic leukodystrophy, Krabbe's disease, adrenoleukodystrophy, Canavan's disease (leukodystrophy), Pelizaeus-Merzbacher disease (leukodystrophy), Alexander's disease (leukodystrophy), and the like); dementia disease (Alzheimer's disease, senile dementia of Alzheimer type, Pick's disease, cerebrovascular dementia, Creutzfeldt-Jakob disease, Parkinson-dementia complex, normal pressure hydrocephalus, progressive supranuclear palsy, and the like); basal nuclei degenerative disease (e. g., Parkinson disease, symptomatic parkinsonism, striatonigral denegeration, Parkinson-dementia complex, Huntington's disease, essential tremor, athetosis, dystonia syndrome (e. g., idiopathic torsion dystonia, local dystonia (spasmodic wryneck, writer's cramp, Meige's disease, and the like), symptomatic dystonia (Hallervorden-Spatz disease, drug-induced dystonia, and the like), Gilles de la Tourette's syndrome, and the like); spinocerebellar degenerative disease (e.g., spinocerebellar degeneration (Shy-Drager syndrome, Machado-Joseph disease, and the like), Louis-Bar syndrome, Bassen-Kornzweig syndrome, Refsum disease, other cerebellar ataxias, and the like); motor neuron diseases (e. g., amyotrophic lateral sclerosis, progressive bulbar amyotrophy (see amyotrophic lateral sclerosis), familial amyotrophic lateral sclerosis, Werdnig-Hoffmann disease, Kugel-

berg-Welander disease, bulbar spinal sclerosis, juvenile one upper limb muscular sclerosis, and the like); tumor diseases of brain and spinal cord (e. g., intracranial tumor, spinal abscess, meningeal carcinoma, and the like); functional diseases (e. g., epilepsy, chronic headache, syncope (see syncope), idiopathic endocranial increased intracranial pressure disease, Meniere disease, narcolepsy, Kleine-Levin syndrome, and the like); toxic and metabolic diseases (e. g., drug intoxication (phenothiazines-derived antipsychotic agent intoxication, sedatives and hypnotics intoxication, antibiotics intoxication, antiparkinson drug, antitumor drug intoxication, β -blocker intoxication, calcium antagonist intoxication, clofibrate intoxication, antiemetic drug intoxication, SMON disease, salicylic acid intoxication, digitalis intoxication, narcotic addiction, and the like), chronic alcoholism (Wernicke encephalopathy, Marchiafava-Bignami syndrome, central pontine myelinolysis, and the like), organic solvent poisoning and pesticide poisoning (e.g., organophosphate compounds poisoning, carbamates poisoning, chloropicrin poisoning, paraquat poisoning, and the like), organophosphate nerve gas poisoning, carbon monoxide poisoning, hydrogen sulfide poisoning, cyanide compound poisoning, mercurial poisoning (metallic mercurial poisoning, inorganomercurial poisoning, organomercurial poisoning, and the like), lead poisoning, tetraethyl lead poisoning, arsenic poisoning, cadmium poisoning, chrome poisoning, manganese poisoning, metal fume fever, sedatives and hypnotics intoxication, salicylic acid intoxication, digitalis intoxication, narcotic addiction, food poisoning (e. g., natural food poisoning (tetradotoxin poisoning, measles shell fish poison food poisoning, diarrhogenic shell fish poison food poisoning, ciguatera, mushroom poisoning, potato-plant poisoning, and the like),

vitamin deficiency (vitamin A deficiency, vitamin B1 deficiency, vitamin B2 deficiency, pellagra, scurvy, vitamin dependency), lipidosis, Gaucher disease, Niemann-Pick disease, and the like), acquired disorders of amino acid metabolism, Wilson disease, amyloidosis, and the like); congenital deformity (Arnold-Chiari malformation, Klippel-Feil syndrome, basilar impression, syringomyelia); neurosis and dermatopathy (e.g., phacomatosis, von-Recklinghausen, tuberous sclerosis, Sturge-Weber, von Hippel Lindau, and the like); spinal diseases (deformity of the spine, herniated intervertebral discs, posterior longitudinal ligament osteosis, and the like), and the like.

[0198]

As used herein, the term "nervous disorder" refers to a disorder of a function, structure, or both of a nerve caused by hereditary relating to development, defects in development, or exogenous factors (e. g., toxins, traumas, diseases, and the like). Examples of nervous disorders include, but are not limited to, peripheral nervous disorders, diabetic nervous disorder, and the like. The peripheral nerve is disordered by various causes. Irrespective of causes, peripheral nervous disorders are collectively called "neropathy". Examples of causes for nervous disorders include hereditary, infection, poisoning, metabolic disorders, allergy, collagen diseases, cancer, vascular disorders, traumas, mechanical pressure, tumor, and the like. In some cases, a cause for a nervous disorder is not identified in clinical situations. The present invention encompasses nervous disorders having unknown causes as subjects to be treated. Examples of nervous disorders include, but are not limited to, parenchymatous neuropathy and interstitial neuropathy. Parenchymatous neuropathy indicates that at least one of neuron, Schwann cell and medially sheath

which substantially constitute the peripheral nerve is affected by a pathogen, and a lesion occurs therein. Intestinal neuropathy refers to disorders in which stroma is affected. Examples of intestinal neuropathy include, but are not limited to, physical pressure, vascular lesion (periarteritis nodosa, collagen diseases, etc.), inflammation, and granulation tissue (e. g., leproma, sarcoidosis and the like). If the metabolism of the whole neuron is disordered, the peripheral portion of a neuron is degenerated; the degeneration progresses toward the cell body; and eventually the nerve cell shrinks (antidromic necrotizing neuropathy). Examples of syndromes of nervous disorders include, but are not limited to, motor disorders, sensory disorders, loss of muscle strength, muscular atrophy, loss of reflex, autonomic disorders, combinations thereof, and the like. The present invention is effective for treatment, prophylaxis and the like of such nervous disorders.

[0199]

As used herein, the term "nervous condition" refers to the degree of the health of a nerve. Such a condition can be represented by various parameters. The present invention makes it possible to determine the condition of a nerve by measuring Pep5, p75, Rho GDI, GT1b, MAG, p21, or the like.

[0200]

As used herein, the term "regeneration" refers to the recovery of injured tissue or organ to the original condition, and is also called pathological regeneration. The body of an organism may lose a part of organs or may be heavily injured by traumas or diseases in its life time. In this case, whether or not the injured organ can regenerate varies among organs (or among animal species). The branch of medicine that permits

organs (or tissue), which cannot naturally regenerate, to regenerate so as to recover the function, is regeneration medicine. Whether or not tissue has regenerated, can be determined based on whether or not the function is improved.

5 Mammals have capability of regenerating tissue and organs to some degree (e.g., regeneration of skin, liver, and blood). However, the tissue of certain organs or the central nervous system, such as heart, lung, brain, and the like has poor ability to regenerate. It has been believed that once such tissue is
10 injured, the function cannot be recovered. Therefore, conventionally, when such an organ is injured, organ transplant is substantially the only measure for the treatment of the organ. In the case of the central nervous system to which transplant is not applicable, substantially no treatment is
15 available.

[0201]

As used herein, the term "nerve regeneration" refers to the recovery of an injured or extinguished nerve.
20 Conventionally, it is believed that nerves, particularly the central nervous system, cannot regenerate in the adult. Once nerves lose their function, it is difficult to regenerate it. Whether or not a nerve has regenerated can be confirmed by assessing motor or sensory ability, axonal regeneration in
25 tissue, or the like.

[0202]

(Gene Therapy)

In a specific embodiment, a nucleic acid containing
30 the nucleic acid sequence of a normal gene of the present invention, or a sequence encoding an antibody or a functional derivative thereof is administered for the purposes of gene therapy for treating, inhibiting, or preventing diseases or

disorders associated with the abnormal expression and/or activity of a polypeptide of the present invention. Gene therapy refers to a therapy performed by administering a nucleic acid, which has been expressed or is capable of being expressed, into subjects. In this embodiment of the present invention, a nucleic acid produces a protein encoded thereby and the protein mediates a therapeutic effect.

[0203]

Any method available in the art for gene therapy may be used in accordance with the present invention. Illustrative methods are described below.

[0204]

See the following review articles for gene therapy: Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); and May, TIBTECH 11(5):155-215(1993). Generally known recombinant DNA techniques used for gene therapy are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

[0205]

Therefore, in the present invention, gene therapy using a gene such as Pep5, p75, Rho GDI, MAG and p21 may be useful.

[0206]

As used herein, the terms "trait" and "phenotype" are used interchangeably to refer to a observable trait, a

detectable trait or other measurable traits of organisms. An example of a trait is a symptom of a disease or sensitivity to a disease. The term "trait" or "phenotype" may be used herein typically to refer to symptoms of breast-related diseases (e.g.,
5 breast cancer), obesity or obesity-related disorders, particularly atherosclerosis, insulin resistance, hypertension, microangiopathy in an obesity individual with type II diabetic, ocular lesion associated with microangiopathy in an obesity individual with type II diabetic, or renal lesion
10 associated with microangiopathy in an obesity individual with type II diabetic, or the morbidity thereof.

[0207]

As used herein, the term "genotype" refers to a genetic
15 structure of an individual organism, and often refers to an allele present in an individual or sample. The term "determine the genotype" of a sample or individual encompasses analysis of the sequence of a specific gene of the individual.

20 [0208]

As used herein, the term "polymorphism" refers to the occurrence of at least two selective genomic sequences or alleles between different genomes or individuals. The term "polymorphism (polymorphic)" refers to a state having the
25 possibility that at least two mutants are found in a specific genomic sequence in individuals. The term "polymorphic site" refers to a gene locus at which such a mutation occurs. Single nucleotide polymorphisms (SNPs) indicate that a nucleotide is replaced with another nucleotide at a polymorphic site.
30 A single nucleotide deletion or insertion can lead to a single nucleotide polymorphism. As used herein, the term "single nucleotide polymorphism" preferably refers to a single nucleotide substitution. In general, two different nu-

cleotides may share a polymorphic site between different individuals. In the present invention, polymorphisms of Pep5, p75, Rho GDI, MAG, p21 and the like are considered to be associated with nervous diseases. In one embodiment, alleles
5 identified by such polymorphism analysis may be effective for regeneration, prophylaxis, diagnosis, treatment, or prognosis.

[0209]

10 (Demonstration of Therapeutic Activity or Prophylactic Activity)

The compounds or pharmaceutical compositions of the present invention are preferably tested *in vitro*, and then *in vivo* for the desired therapeutic or prophylactic activity,
15 prior to use in humans. For example, *in vitro* assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or
20 tissue sample can be determined utilizing techniques known to those of skill in the art (including, but not limited to, cell lysis assays). In accordance with the present invention, *in vitro* assays which can be used to determine whether administration of a specific compound is indicated, include
25 *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

30 [0210]

(Therapeutic/Prophylactic Administration and Composition)

The present invention provides methods of treatment,

inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the present invention. In a preferred aspect, the compound is substantially purified (e. g., substantially free from
5 substances that limit its effect or produce undesired side-effects).

[0211]

As used herein, term "amount effective for diagnosis,
10 prophylaxis, treatment, or prognosis" refers to an amount which is recognized as therapeutically effective for diagnosis, prophylaxis, treatment (or therapy), or prognosis. Such an amount can be determined by those skilled in the art using techniques well known in the art with reference to various
15 parameters.

[0212]

Animals targeted by the present invention include any organism as long as it has a nervous system or its analogous
20 system (e. g., animals (e. g., vertebrates, invertebrate)). Preferably, the animal is a vertebrate (e. g., Myxiniiformes, Petronyzoniformes, Chondrichthyes, Osteichthyes, amphibian, reptilian, avian, mammalian, and the like), more preferably mammalian (e. g., monotremata, marsupialia, edentate,
25 dermoptera, chiroptera, carnivore, insectivore, proboscidea, perissodactyla, artiodactyla, tubulidentata, pholidota, sirenia, cetacean, primates, rodentia, lagomorpha, and the like). Illustrative examples of a subject include, but are not limited to, animals, such as cattle, pig, horse, chicken,
30 cat, dog, and the like. More preferably, cells derived from Primates (e. g., chimpanzee, Japanese monkey, human) are used. Most preferably, cells derived from a human are used.

[0213]

When a nucleic acid molecule or polypeptide of the present invention is used as a medicament, the medicament may further comprise a pharmaceutically acceptable carrier. Any
5 pharmaceutically acceptable carrier known in the art may be used in the medicament of the present invention.

[0214]

Examples of a pharmaceutical acceptable carrier or a
10 suitable formulation material include, but are not limited to, antioxidants, preservatives, colorants, flavoring agents, diluents, emulsifiers, suspending agents, solvents, fillers, bulky agents, buffers, delivery vehicles, and/or pharmaceutical adjuvant. Representatively, a medicament of the
15 present invention is administered in the form of a composition comprising a polypeptide or a polynucleotide, such as Pep5, p75, Rho GDI, MAG and p21, or a variant or derivative thereof with at least one physiologically acceptable carrier, excipient or diluent. For example, an appropriate vehicle may be
20 injection solution, physiological solution, or artificial cerebrospinal fluid, which can be supplemented with other substances which are commonly used for compositions for parenteral delivery.

25 [0215]

Acceptable carriers, excipients or stabilizers used herein preferably are nontoxic to recipients and are preferably inert at the dosages and concentrations employed, and preferably include phosphate, citrate, or other organic acids;
30 ascorbic acid, α -tocopherol; low molecular weight polypeptides; proteins (e. g., serum albumin, gelatin, or immunoglobulins); hydrophilic polymers (e. g., polyvinylpyrrolidone); amino acids (e. g., glycine, glutamine,

asparagine, arginine or lysine); monosaccharides, disaccharides, and other carbohydrates (including glucose, mannose, or dextrans); chelating agents (e. g., EDTA); sugar alcohols (e. g., mannitol or sorbitol); salt-forming counterions (e. g., sodium); and/or nonionic surfactants (e. g., Tween, pluronics or polyethylene glycol (PEG)).

[0216]

Examples of appropriate carriers include neutral buffered saline or saline mixed with serum albumin. Preferably, the product is formulated as a lyophilizing agent using appropriate excipients (e. g., sucrose). Other standard carriers, diluents, and excipients may be included as desired. Other exemplary compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute therefor.

[0217]

Hereinafter, commonly used preparation methods of the medicament of the present invention will be described. Note that animal drug compositions, quasi-drugs, marine drug compositions, food compositions, cosmetic compositions, and the like are prepared using known preparation methods.

[0218]

The polypeptide, polynucleotide and the like of the present invention can be mixed with a pharmaceutically acceptable carrier and can be orally or parenterally administered as solid formulations (e. g., tablets, capsules, granules, abstracts, powders, suppositories, and the like) or liquid formulations (e. g., syrups, injections, suspensions, solutions, spray agents and the like). Examples of pharmaceutically acceptable carriers include excipients,

lubricants, binders, disintegrants, disintegration inhibitors, absorption promoters, adsorbers, moisturizing agents, solubilizing agents, stabilizers and the like in solid formulations; and solvents, solubilizing agents, suspending agents, isotonic agents, buffers, soothing agents and the like in liquid formulations. Additives for formulations, such as antiseptics, antioxidants, colorants, sweeteners, and the like can be optionally used. The composition of the present invention can be mixed with substances other than the polynucleotide, polypeptide, and the like of the present invention. Examples of parenteral routes of administration include, but are not limited to, intravenous injection, intramuscular injection, intranasal, rectum, vagina, transdermal, and the like.

[0219]

Examples of excipients in solid formulations include glucose, lactose, sucrose, D-mannitol, crystallized cellulose, starch, calcium carbonate, light silicic acid anhydride, sodium chloride, kaolin, urea, and the like.

[0220]

Examples of lubricants in solid formulations include, but are not limited to, magnesium stearate, calcium stearate, boric acid powder, colloidal silica, talc, polyethylene glycol, and the like.

[0221]

Examples of binders in solid formulations include, but are not limited to, water, ethanol, propanol, saccharose, D-mannitol, crystallized cellulose, dextran, methylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, carboxymethylcellulose, starch solution, gelatin solution,

polyvinylpyrrolidone, calciumphosphate, potassiumphosphate, shellac, and the like.

[0222]

5 Examples of disintegrants in solid formulations include, but are not limited to, starch, carboxymethyl-cellulose, carboxymethylcellulose calcium, agar powder, laminarin powder, croscarmellose sodium, carboxymethyl starch sodium, sodium alginate, sodium hydrocarbonate,
10 calcium carbonate, polyoxyethylene sorbitan fatty acid esters, sodium lauryl sulfate, starch, monoglyceride stearate, lactose, calcium glycolate cellulose, and the like.

[0223]

15 Examples of disintegration inhibitors in solid formulations include, but are not limited to, hydrogen-added oil, saccharose, stearin, cacao butter, hydrogenated oil, and the like.

20 [0224]

 Examples of absorption promoters in solid formulations include, but are not limited to, quaternary ammonium salts, sodium lauryl sulfate, and the like.

25 [0225]

 Examples of absorbers in solid formulations include, but are not limited to, starch, lactose, kaolin, bentonite, colloidal silica, and the like.

30 [0226]

 Examples of moisturizing agents in solid formulations include, but are not limited to, glycerin, starch, and the like.

[0227]

Examples of solubilizing agents in solid formulations include, but are not limited to, arginine, glutamic acid, aspartic acid, and the like.

[0228]

Examples of stabilizers in solid formulations include, but are not limited to, human serum albumin, lactose, and the like.

[0229]

When tablets, pills, and the like are prepared as solid formulations, they may be coated, if necessary, with film of a substance dissolvable in the stomach or the intestine (saccharose, gelatin, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, and the like). Tablets include those optionally with a typical coating (e.g., dragees, gelatin coated tablets, enteric coated tablets, film coated tablets or double tablets, multilayer tablets, and the like). Capsules include hard capsules and soft capsules. When tablets are molded into the form of suppository, higher alcohols, higher alcohol esters, semi-synthesized glycerides, can be added in addition to the above-described additives, although not limited to them.

[0230]

Preferable examples of solvents in liquid formulations include injection solutions, alcohols, propyleneglycol, macrogol, sesami oil, corn oil, and the like.

[0231]

Preferrable examples of solubilizing agents in liquid

formulations include, but are not limited to, polyethyleneglycol, propyleneglycol, D-mannitol, benzyl benzoate, ethanol, trisaminomethane, cholesterol, triethanolamine, sodium carbonate, sodium citrate, and the like.

5

[0232]

Preferable examples of suspending agents in liquid formulations include surfactants such as stearyltriethanolamine, sodium lauryl sulfate, lauryl amino propionic acid,
10 lecithin, benzalkonium chloride, benzethonium chloride, glycerin monostearate, and the like, hydrophilic macromolecule such as polyvinyl alcohol, polyvinylpyrrolidone, carboxymethylcellulose sodium, methylcellulose, hydroxymethylcellulose, hydroxyethylcellulose, hydroxyethylcellulose,
15 cellulose, hydroxypropylcellulose, and the like.

[0233]

Preferable examples of isotonic agents in liquid formulations include, but are not limited to, sodium chloride,
20 glycerin, D-mannitol, and the like.

[0234]

Preferable examples of buffers in liquid formulations include, but are not limited to, phosphate, acetate, carbonate,
25 citrate, and the like.

[0235]

Preferable examples of soothing agents in liquid formulations include, but are not limited to, benzyl alcohol,
30 benzalkonium chloride, procaine hydrochloride, and the like.

[0236]

Preferable examples of antiseptics in liquid for-

mulations include, but are not limited to, parahydroxybenzoate ester, chlorobutanol, benzyl alcohol, 2-phenylethylalcohol, dehydroacetic acid, sorbic acid, and the like.

5 [0237]

Preferable examples of antioxidants in liquid formulations include, but are not limited to, sulfite, ascorbic acid, α -tocopherol, cysteine, and the like.

10 [0238]

When liquid agents and suspensions are prepared as injections, they are sterilized and are preferably isotonic with the blood. Typically, these agents are made aseptic by filtration using a bacteria-contained filter or the like, mixing with a bactericide, irradiation, or the like. Following these treatment, these agents may be made solid by lyophilization or the like. Immediately before use, sterile water or sterile injection diluent (lidocaine hydrochloride aqueous solution, physiological saline, glucose aqueous solution, ethanol or a mixture solution thereof, and the like) may be added.

[0239]

The medicament composition of the present invention may further comprise a colorant, a preservative, a flavor, an aroma chemical, a sweetener, or other drugs.

[0240]

The medicament of the present invention may be administered orally or parenterally. Alternatively, the medicament of the present invention may be administered intravenously or subcutaneously. When systemically administered, the medicament for use in the present invention

may be in the form of a pyrogen-free, pharmaceutically acceptable aqueous solution. The preparation of such pharmaceutically acceptable compositions, with due regard to pH, isotonicity, stability and the like, is within the skill
5 of the art. Administration methods may be herein oral, parenteral administration (e.g., intravenous, intramuscular, subcutaneous, intradermal, to mucosa, intrarectal, vaginal, topical to an affected site, to the skin, and the like). A prescription for such administration may be provided in any
10 formulation form. Such a formulation form includes liquid formulations, injections, sustained preparations, and the like.

[0241]

15 The medicament of the present invention may be prepared for storage by mixing a sugar chain composition having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Japanese Pharmacopeia ver. 14 or the latest version; Remington's
20 Pharmaceutical Sciences, 18th Edition, A. R. Gennaro, ed., Mack Publishing Company, 1990; and the like), in the form of lyophilized cake or aqueous solutions.

[0242]

25 Various delivery systems are known and can be used to administer a compound of the present invention (e.g., liposomes, microparticles, microcapsules and the like). Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous,
30 intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route (e.g., by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal

and intestinal mucosa, and the like) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the present invention into the central nervous system by any suitable route (including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir). Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0243]

In a specific embodiment, it may be desirable to administer a polypeptide, polynucleotide or composition of the present invention locally to the area in need of treatment (e.g., the central nervous system, the brain, or the like); this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application (e.g., in conjunction with a wound dressing after surgery), by injection, by means of a catheter, by means of a suppository, or by means of an implant (the implant is a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers). Preferably, when administering a protein, including an antibody, of the present invention, care must be taken to use materials which does not absorb the protein.

[0244]

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249: 1527-1533. (1990); Treat et al., Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein

and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

[0245]

5 In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14: 201 (1987); Buchwald et al., Surgery 88: 507 (1980); Saudek et al., N. Engl. J. Med. 321: 10 574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and 15 Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23: 61 (1983); see also Levy et al., Science 228: 190 (1985); During et al., Ann. Neurol. 25: 351 (1989); Howard et al., J. Neurosurg. 71: 105 (1989)).

20 [0246]

 In yet another embodiment, a controlled release system can be placed in proximity to the therapeutic target, i. e., the brain, thus requiring only a fraction of the systemic dose (see, e. g., Goodson, in Medical Applications of Controlled 25 Release, *supra*, vol. 2, pp. 115-138 (1984)).

[0247]

 Other controlled release systems are discussed in the review by Langer (Science 249: 1527-1533 (1990)).

30

[0248]

 The amount of a compound used in the treatment method of the present invention can be easily determined by those

skilled in the art with reference to the purpose of use, a target disease (type, severity, and the like), the patient's age, weight, sex, and case history, the form or type of the cells, and the like. The frequency of the treatment method of the present invention which is applied to a subject (patient) is also determined by the those skilled in the art with respect to the purpose of use, a target disease (type, severity, and the like), the patient's age, weight, sex, and case history, the progression of the therapy, and the like. Examples of the frequency include once per day to several months (e.g., once per week to once per month). Preferably, administration is performed once per week to month with reference to the progression.

15 [0249]

The doses of the polypeptides, polynucleotides or the like of the present invention vary depending on the subject's age, weight and condition or an administration method, or the like, including, but not limited to, ordinarily 0.01 mg to 10 g per day for an adult in the case of oral administration, preferably 0.1 mg to 1 g, 1 mg to 100 mg, 0.1 mg to 10 mg, and the like; in the case of parenteral administration, 0.01 mg to 1 g, preferably 0.01 mg to 100 mg, 0.1 mg to 100 mg, 1 mg to 100 mg, 0.1 mg to 10 mg, and the like.

25

[0250]

As used herein, the term "administer" means that the polypeptides, polynucleotides or the like of the present invention or pharmaceutical compositions containing them are administered either alone or in combination with other therapeutic agents. Combinations may be administered either concomitantly as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations

in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously (e.g., as through separate intravenous lines into the same individual).

- 5 "Combination" administration further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0251]

- 10 As used herein, "instructions" describe a method of administering a medicament of the present invention, a method for diagnosis, or the like for persons who administer or persons who diagnose, such as physicians, patients, and the like (may be a patient in person). The instructions describe a statement
15 indicating a method for administering a diagnostic, medicament, or the like of the present invention. The instructions are prepared in accordance with a format defined by an authority of a country in which the present invention is practiced (e. g., Health, Labor and Welfare Ministry in
20 Japan, Food and Drug Administration (FDA) in U.S., and the like), explicitly describing that the instructions are approved by the authority. The instructions are so-called package insert and are typically provided in paper media, but the instructions are not so limited and may be provided in
25 the form of electronic media (e. g., homepages (web sites) and electronic mails provided on the Internet).

[0252]

- 30 The judgment of termination of treatment with a method of the present invention may be supported by a result of a standard clinical laboratory using commercially available assays or instruments or extinction of a clinical symptom characteristic to a disease (e. g., a neurological disease)

associated with Pep5, p75, Rho GDI, MAG, GT1b and p21. Treatment can be resumed by the relapse of a disease (e. g., a neurological disease) associated with Pep5, p75, Rho GDI, MAG, GT1b and p21.

5 The present invention also provides a pharmaceutical package or kit comprising one or more containers loaded with one or more pharmaceutical compositions. A notice in a form defined by a government agency which regulates the production, use or sale of pharmaceutical products or biological products
10 may be arbitrarily attached to such a container, representing the approval of the government agency relating to production, use or sale with respect to administration to a human.

[0253]

15 (Detailed Description)

 The present inventors' studies demonstrated that the association of p75^{NTR} with Rho GDI is enhanced by MAG and Nogo. As p75^{NTR} has an ability to release RhoA from Rho GDI *in vitro*, activation of RhoA by MAG and Nogo through p75^{NTR} may be
20 attributable, at least partly, to Rho GDI displacement. The release of Rho from Rho GDI is an important step allowing the activation by guanine nucleotide exchange agents and membrane association of the GTP-bound form of Rho. As p75^{NTR} itself may not mediate the process of guanine nucleotide exchange, some
25 Rho guanine nucleotide exchange agents might co-operate with p75^{NTR}, which is one of the issues to be addressed in the future. It is noted that another Rho GDI displacement agent, ezrin/radixin/moesin, also induces activation of RhoA in Swiss 3T3 cells, which is similar to our findings that p75^{NTR}
30 activates RhoA.

[0254]

 There is growing evidence that p75^{NTR} has a key role

in axon guidance or growth during the developmental stage (Cited Reference 1). Axon outgrowth from spinal motor neurons or forelimb motor neurons in mice carrying a mutation in p75^{NTR} is significantly retarded in vivo (Cited References 2 and 17).

5 This phenotype may be attributable to ligand binding to p75^{NTR}, as the chick ciliary neurons, which express p75^{NTR} but not TrkA, extend neurites in response to NGF. Contrary to these observations, aberrant axonal elongation is observed in myelin-rich areas where these axons would normally not grow

10 in mice carrying a mutation in p75^{NTR} (Cited Reference 18). In line with this finding, all the myelin-derived inhibitors of neurite outgrowth identified so far inhibit growth that is dependent on p75^{NTR} (Cited References 5, 6 and 7). The findings of the present inventors suggest that these effects

15 may result from the Rho GDI displacement activity of p75^{NTR}. In addition, axon pathfinding errors of p75^{NTR}-expressing neurons are prominent among the phenotypes observed in mice carrying a mutation in p75^{NTR} (including mistargeting of sympathetic and cortical subplate axons) (Cited References 19

20 and 20). As Rho seems to be involved in the regulation of axon pathfinding in the developmental stages, it is possible that the mistargeting in the absence of p75^{NTR} may be attributable to the failure of appropriate regulation of Rho activity. Interestingly, a recent report suggests a role of Rho GDI in

25 spatial and temporal activation of the downstream pathway of Rac1 (Cited Reference 21). Although Rho GDI associates with Rac1 and blocks effector binding, release of Rac1 from Rho GDI at specific regions where integrin localizes allows Rac1 to bind its effectors. Thus, Rho GDI is suggested to confer

30 spatially restricted regulation of Rho GTPases-effectors interaction. In future studies, it will be interesting to test the hypothesis that spatial control of Rho signaling regulated by Rho GDI may participate in the axon pathfindings.

[0255]

A short isoform of p75^{NTR} has been found which lacks three of the four cysteine-rich repeats in the extracellular
5 ligand-binding domain but has the intact intracellular domain (Cited Reference 22). The cells from mice bearing a targeted disruption of the third exon of the p75^{NTR} gene express this short isoform of p75^{NTR} (Cited Reference 23), but are insensitive to inhibitory molecules (Cited References 5, 6 and
10 7). As the present inventors' data show that Pep5 did not affect the neurite outgrowth of the neurons which express the short isoform but not the full-length p75^{NTR} (Fig. 5b), the short isoform might not act as a regulator of the neurite outgrowth.

15 [0256]

As such a short isoform is a component constituting an interacellular domain, p75 comprising a component containing an extracellular domain may be used in a preferred embodiment.

20 [0257]

It is now well established that axons of the adult central nervous system are capable of only a limited amount of regrowth after injury, and that an unfavorable environment
25 plays major a role in the lack of regeneration. Much of the axon growth inhibitory effects are associated with myelin. Identification of the myelin-derived inhibitors led to confirmation of the present inventors' recognition about the molecular mechanisms of the biological activities. Therefore,
30 it is now an important issue to explore strategies to overcome the inhibitory signals. The present inventors note that. Pep5 seems to specifically inhibit the action mediated by myelin-derived inhibitors, as Pep5 did not inhibit the

NGF-induced promotion of the neurite outgrowth from hippocampal neurons (data not shown) or the cell death of superior cervical ganglion neurons treated with 100 ng/ml BDNF (data not shown). Specific inhibition of myelin-associated inhibitor effects may provide a practical therapeutic agent for injuries to the central nervous system.

[0258]

(Best Mode for Carrying Out the Present Invention)

Hereinafter, preferred embodiments of the present invention will be described. Embodiments provided below are provided for better understanding of the present invention. It will be understood that the scope of the present invention is not limited to the following description. Therefore, it is apparent that those skilled in the art can appropriately modify the present invention without departing from the spirit or scope of the present invention by referencing the description of the specification.

[0259]

(Pep5 in the Polypeptide Form)

In one aspect, the present invention provides a composition comprising a Pep5 polypeptide for regenerating nerves, and a composition comprising a Pep5 polypeptide for treatment, prophylaxis, diagnosis or prognosis of nervous diseases, nervous disorders or nervous conditions. An effective amount of the composition for regeneration, diagnosis, prophylaxis, treatment, or prognosis can be determined by those skilled in the art based on the disclosures of the present specification using techniques well known in the art with reference to various parameters. For example, such an amount can be determined by those skilled in the art with reference to the purpose of use, a target disease (type,

severity, and the like), the patient's age, weight, sex and case history, the form or type of the cells, and the like (see Shinkei-Naika Chiryō Gaido [Guidance to Treatments in Neurological Internal Medicine], Norio Ogawa, Chugai-Igaku 5 1994). In the present invention, it was revealed that regeneration of nerves occurs due to inhibition of neurite outgrowth being disrupted by blocking of the p75 signal transduction pathway (by Pep5). The effect of nerve re-10 generation by blocking of a signal transduction pathway has not been conventionally known. Therefore, the present invention provides an effect more excellent than the prior art.

[0260]

15 In one embodiment of the present invention, Pep5 or fragments or variants thereof comprise (a) a polypeptide consisting of an amino acid sequence as set forth in SEQ ID NO. 2; (b) a polypeptide having at least one mutation selected from the group consisting of one or more amino acid sub-20 stitutions, additions and deletions in the amino acid sequence as set forth in SEQ ID NO: 2 and having a biological activity; (c) a polypeptide encoded by a splice variant or an allelic variant; or (d) a polypeptide which is a species homolog of the amino acid sequence as set forth in SEQ ID NO: 2; or (e)25 a polypeptide having an amino acid sequence having at least 70% homology to any one of the polypeptides described in (a) to (d), and having biological activity.

[0261]

30 In one preferred embodiment, the number of substitutions, additions and deletions described in (b) above may be limited, and is preferably, for example, 50 or less, 40 or less, 30 or less, 20 or less, 15 or less, 10 or less,

9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. The number of substitutions, additions and deletions is preferably smaller, but may be large as long as the biological activity is maintained (preferably, the activity is similar to or substantially the same as that of Pep5).

[0262]

In another preferred embodiment, the allelic variant in the above (c) preferably has a homology of at least 99% with the amino acid sequence as set forth in SEQ ID NO: 2.

[0263]

In another preferred embodiment, the above species homolog preferably can be identified as described above in the specification and has at least about 30% of homology with the amino acid sequence as set forth in SEQ ID NO: 2.

[0264]

In another preferred embodiment, the biological activity possessed by the variant polypeptide described in (e) above includes, but not limited to, for example, an interaction with an antibody specific to the polypeptide consisting of the amino acid sequence as set forth in SEQ ID NO. 2 or a fragment thereof; an interaction with the p75 polypeptide; and the like.

[0265]

In a preferred embodiment, the above-described homology to any one of the polypeptides described in (a) to (d) above may be at least about 80%, more preferably at least about 90%, even more preferably at least about 98%, and most preferably at least about 99%.

[0266]

The polypeptide of the present invention typically has a sequence of at least 3 contiguous amino acids. The amino acid length of the polypeptide of the present invention may be short as long as the peptide is suitable for an intended application, but preferably a longer sequence may be used. Therefore, the amino acid length may be preferably at least 4, more preferably at least 5, at least 6, at least 7, at least 8, at least 9 and at least 10, even more preferably at least 15, and still even more preferably at least 20. These lower limits of the amino acid length may be present between the above-specified numbers (e.g., 11, 12, 13, 14, 16, and the like) or above the above-specified numbers (e.g., 21, 22, 30, and the like). The upper limit of the length of the polypeptide of the present invention may be greater than or equal to the full length of the sequence as set forth in SEQ ID NO. 2 as long as the peptide is capable of interacting with a given agent.

[0267]

In one embodiment, the Pep5 polypeptide or fragments or variants thereof comprise the whole amino acid sequence as set forth in SEQ ID NO. 2. More preferably, the Pep5 or fragments or variants thereof consist of the whole amino acid sequence as set forth in SEQ ID NO. 2.

[0268]

In one embodiment, nervous diseases, disorders or conditions to be treated are exemplified herein elsewhere and include, for example, Alzheimer's disease, spinal cord injury, cerebrovascular disorder, brain injury, and the like. Preferably, a nervous disease, disorder or condition intended

to be treated by the composition of the present invention may be Alzheimer's disease. In another preferred embodiment, nervous diseases, disorders or conditions intended to be treated by the composition of the present invention may be
5 spinal cord injury, cerebrovascular disorder, and brain injury.

[0269]

(Pep5 in the Nucleic Acid Form)

10 In one aspect, the present invention provides a composition comprising a nucleic acid molecule encoding the Pep5 polypeptide for regenerating nerves, and a composition comprising a nucleic acid molecule encoding the Pep5 polypeptide for treatment, prophylaxis, diagnosis or
15 prognosis of nervous diseases, nervous disorders or nervous conditions. An effective amount of the composition for regeneration, diagnosis, prophylaxis, treatment, or prognosis can be determined by those skilled in the art based on the disclosures of the present specification using
20 techniques well known in the art with reference to various parameters. For example, such an amount can be determined by those skilled in the art with reference to the purpose of use, a target disease (type, severity, and the like), the patient's age, weight, sex and case history, the form or type of the
25 cells, and the like (see Shinkei-Naika Chiryō Gaido [Guidance to Treatments in Neurological Internal Medicine]; Norio Ogawa, Chugai-Igaku 1994). In the present invention, it was revealed that regeneration of nerves occurs due to inhibition of neurite outgrowth being disrupted by blocking of the p75 signal
30 transduction pathway (by Pep5). The effect of nerve regeneration by blocking of a signal transduction pathway has not been conventionally known. Therefore, the present invention provides an effect more excellent than the prior

art.

[0270]

In one embodiment of the present invention, the nucleic
5 acid molecule encoding Pep5 or fragments or variants thereof
comprise (a) a polynucleotide having the base sequence as set
forth in SEQ ID NO. 1 or a fragment thereof; (b) a polynucleotide
encoding a polypeptide having an amino acid sequence as set
10 forth in SEQ ID NO. 2 (CFFRGGFFNHNPRYC) or a fragment thereof;
(c) a polynucleotide encoding a variant polypeptide having
the amino acid sequence as set forth in SEQ ID NO. 2 having
at least one mutation selected from the group consisting of
one or more amino acid substitutions, additions and deletions,
and having biological activity; (d) a polynucleotide which
15 is a splice variant or an allelic variant of the base sequence
as set forth in SEQ ID NO: 1; (e) a polynucleotide encoding
a specie homolog of a polypeptide consisting of the amino acid
sequence as set forth in SEQ ID NO: 2; (f) polynucleotide
hybridizable to any one of the polynucleotides described in
20 (a) to (e) under stringent conditions and encoding a
polypeptide having biological activity; or (g) a polynu-
cleotide consisting of a base sequence having at least 70%
identity to any one of the polynucleotides described in (a)
to (e) or a complementary sequence thereof and encoding a
25 polypeptide having biological activity.

[0271]

In one preferred embodiment, the number of sub-
stitutions, additions and deletions described in (c) above
30 may be limited to, for example, preferably 50 or less, 40 or
less, 30 or less, 20 or less, 15 or less, 10 or less, 9 or
less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less,
3 or less, or 2 or less. The number of substitutions, additions

and deletions is preferably small, but may be large as long as the biological activity is maintained (preferably, the activity is similar to or substantially the same as that of Pep5).

5

[0272]

In another preferred embodiment, the biological activity possessed by the above-described variant polypeptide includes, but is not limited to, for example, an interaction with an antibody specific to the polypeptide having the amino acid sequence as set forth in SEQ ID NO. 2 or a fragment thereof; an interaction with p75; modulation of the functional regulation of Rho GDI by p75; and the like. These activities can be measured by, for example, immunological assays, phosphorylation quantification, or the like.

[0273]

In another preferred embodiment, an allelic variant advantageously has a homology of at least about 99% with the nucleic acid sequence as set forth in SEQ ID NO: 1.

[0274]

When there is a gene sequence database of a species, the above species homolog can be identified by searching the database using Pep5 of the present invention as a query sequence. Alternatively, the above species homolog can be identified by screening a gene library of the species using whole or a part of Pep5 of the present invention as a probe or a primer. Such identification methods are well known in the art and are described in references described herein. The species homolog preferably has, for example, a homology of at least about 30% with the nucleic acid sequence as set forth in SEQ ID NO: 1.

[0275]

In a preferred embodiment, the identity to any one of the polynucleotides described in (a) to (e) above or a
5 complementary sequence thereof may be at least about 80%, more preferably at least about 90%, even more preferably at least about 98%, and most preferably at least about 99%.

[0276]

10 In a preferred embodiment, the nucleic acid molecule of the present invention encoding Pep5 or fragments and variants thereof may have a length of at least 8 contiguous nucleotides. The appropriate nucleotide length of the nucleic acid molecule of the present invention may vary depending on
15 the purpose of use of the present invention. More preferably, the nucleic acid molecule of the present invention may have a length of at least 10 contiguous nucleotides, even more preferably at least 15 contiguous nucleotides, and still even more preferably at least 20 contiguous nucleotides. These lower
20 limits of the nucleotide length may be present between the above-specified numbers (e.g., 9, 11, 12, 13, 14, 16, and the like) or above the above-specified numbers (e.g., 21, 22, 30, and the like). The upper limit of the length of the polypeptide of the present invention may be greater than or equal to the
25 full length of the sequence as set forth in SEQ ID NO. 1 as long as the polynucleotide can be used for the intended purpose (e.g. marker, primer, and probe). Alternatively, when the nucleic acid molecule of the present invention is used as a primer, the nucleic acid molecule typically may have a
30 nucleotide length of at least about 8, preferably a nucleotide length of about 10. When used as a probe, the nucleic acid molecule typically may have a nucleotide length of at least about 15, and preferably a nucleotide length about 17.

[0277]

In one embodiment, the nucleic acid molecule encoding Pep5 or fragments or variants thereof comprise the whole
5 nucleic acid sequence as set forth in SEQ ID NO. 1. More preferably, the nucleic acid molecule encoding Pep5 or fragments or variants thereof consist of the whole nucleic acid sequence as set forth in SEQ ID NO. 1.

10 [0278]

In one embodiment, nervous diseases, disorders or conditions to be treated include, for example, Alzheimer's disease, spinal cord injury, cerebrovascular disorder, brain injury, and the like. Preferably, a nervous disease, disorder
15 or condition intended to be treated by the composition of the present invention may be Alzheimer's disease. In another preferred embodiment, nervous diseases, disorders or conditions intended to be treated by the composition of the present invention may be spinal cord injury, cerebrovascular
20 disorder, and brain injury.

[0279]

(Agent Specifically Interacting with p75 in the Polypeptide Form)

25 In one aspect, the present invention provides a composition comprising an agent capable of specifically interacting with a p75 polypeptide for regenerating nerves, and a composition comprising an agent capable of specifically
interacting with a p75 polypeptide for treatment, prophylaxis,
30 diagnosis or prognosis of nervous diseases, nervous disorders or nervous conditions. An effective amount of the composition for regeneration, diagnosis, prophylaxis, treatment, or prognosis can be determined by those skilled in the art based

on the disclosures of the present specification using techniques well known in the art with reference to various parameters. For example, such an amount can be determined by those skilled in the art with reference to the purpose of use, a target disease (type, severity, and the like), the patient's age, weight, sex and case history, the form or type of the cells, and the like (see Shinkei-Naika Chiryo Gaido [Guidance to Treatments in Neurological Internal Medicine], Norio Ogawa, Chugai-Igaku 1994). In the present invention, it was revealed that regeneration of nerves occurs due to inhibition of neurite outgrowth being disrupted by blocking of the p75 signal transduction pathway (by the agent capable of specifically interacting with p75). The effect of nerve regeneration by blocking of a signal transduction pathway has not been conventionally known. Therefore, the present invention provides an effect more excellent than the prior art.

[0280]

In one embodiment of the present invention, the agent of the present invention may be an agent capable of specifically interacting with (a) a polypeptide having an amino acid sequence as set forth in SEQ ID NO: 4 or a fragment thereof; (b) a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 4 having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions and deletions, and having biological activity; (c) a polypeptide encoded by a splice variant or allelic variant of a base sequence as set forth in SEQ ID NO. 3 or 16; (d) a polypeptide which is a species homolog of the amino acid sequence as set forth in SEQ ID NO. 4; or (e) a polypeptide having an amino acid sequence having at least 70% homology to any one of the polypeptides described in (a) to (d), and having, biological activity.

[0281]

In one preferred embodiment, the number of substitutions, additions and deletions described in (b) above
5 may be limited to, for example, preferably 50 or less, 40 or less, 30 or less, 20 or less, 15 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. The number of substitutions, additions and deletions is preferably small, but may be large as long
10 as the biological activity is maintained (preferably, the activity is similar to or substantially the same as that of a product of the p75 gene).

[0282]

15 In a preferred embodiment, an agent of the present invention is selected from the group consisting of a nucleic acid molecule, a polypeptide, lipid, a sugar chain, organic low molecule, and a composite molecule thereof.

20 [0283]

In another preferred embodiment, the allelic variant described in (c) above preferably has at least 99% homology to the amino acid sequence as set forth in SEQ ID NO. 4.

25 [0284]

In another preferred embodiment, the above-described species homolog can be identified as described above and preferably has at least about 30% homology to the amino acid sequence as set forth in SEQ ID NO. 4.

30

[0285]

In another preferred embodiment, the biological activity possessed by the variant polypeptide described in

(e) above includes, but is not limited to, for example, an interaction with an antibody specific to the polypeptide having the amino acid sequence as set forth in SEQ ID NO. 4 or a fragment thereof; an interaction with the Rho GDI polypeptide; and the like.

[0286]

In a preferred embodiment, the above-described homology to any one of the polypeptides described in (a) to (d) above may be at least about 80%, more preferably at least about 90%, even more preferably at least about 98%, and most preferably at least about 99%.

[0287]

The polypeptide with which the agent of the present invention specifically interacts typically has a sequence of at least 3 contiguous amino acids. The amino acid length of the polypeptide of the present invention may be short as long as the peptide is suitable for an intended application, but preferably a longer sequence may be used. Therefore, the amino acid length may be preferably at least 4, more preferably at least 5, at least 6, at least 7, at least 8, at least 9 and at least 10, even more preferably at least 15, and still even more preferably at least 20. These lower limits of the amino acid length may be present between the above-specified numbers (e. g., 11, 12, 13, 14, 16, and the like) or above the above-specified numbers (e. g., 21, 22, ...30, and the like). The upper limit of the length of the polypeptide of the present invention may be greater than or equal to the full length of the sequence as set forth in SEQ ID NO. 4 as long as the peptide is capable of interacting with a given agent.

[0288]

In a preferred embodiment, the agent of the present invention is selected from the group consisting of a nucleic acid molecule, a polypeptide, a lipid, a sugar chain, an organic low molecule and a composite molecule thereof. More preferably, the agent of the present invention is antibody or a derivative thereof (e. g., a single chain antibody). Therefore, the agent of the present invention can be used as a probe.

[0289]

In one embodiment, the p75 polypeptide or fragments or variants thereof comprise amino acids 273 to 427 of SEQ ID NO: 4 or amino acids 274 to 425 of SEQ ID NO. 17. More preferably, the p75 or fragments or variants thereof consist of amino acids 393 to 408 of SEQ ID NO: 4 or amino acids 391 to 406 of SEQ ID NO: 17.

[0290]

In one embodiment, nervous diseases, disorders or conditions to be treated include, for example, Alzheimer's disease, spinal cord injury, cerebrovascular disorder, brain injury, and the like. Preferably, a nervous disease, disorder or condition intended to be treated by the composition of the present invention may be Alzheimer's disease. In another preferred embodiment, nervous diseases, disorders or conditions intended to be treated by the composition of the present invention may be spinal cord injury, cerebrovascular disorder, and brain injury.

[0291]

In a preferred embodiment, the agent of the present invention may be advantageously labeled or capable of being bound to a label. When labeled, various states which can be measured using the agent of the present invention can be

directly and/or readily measured. Any label can be used as long as it can be identified. Examples of a label include, but are not limited to, a fluorescent label, a chemically light emitting label, a radiolabel, and the like. Alternatively, when the agent interacts with an antibody or the like in an immune reaction, a system which is commonly used in an immune reaction, such as biotin-streptavidin may be used.

[0292]

(Agent Interacting with p75 Polypeptide in the Nucleic Acid Form)

In one aspect, the present invention provides a composition comprising an agent capable of specifically interacting with a nucleic acid molecule encoding the p75 polypeptide for regenerating nerves, and a composition comprising an agent capable of specifically interacting with a nucleic acid molecule encoding the p75 polypeptide for treatment, prophylaxis, diagnosis or prognosis of nervous diseases, nervous disorders or nervous conditions. An effective amount of the composition for regeneration, diagnosis, prophylaxis, treatment, or prognosis can be determined by those skilled in the art based on the disclosures of the present specification using techniques well known in the art with reference to various parameters. For example, such an amount can be determined by those skilled in the art with reference to the purpose of use, a target disease (type, severity, and the like), the patient's age, weight, sex and case history, the form or type of the cells, and the like (see Shinkei-Naika Chiryō Gaido [Guidance to Treatments in Neurological Internal Medicine], Norio Ogawa, Chugai-Igaku 1994). In the present invention, it was revealed that regeneration of nerves occurs due to inhibition of neurite outgrowth being disrupted by blocking of the p75 signal

transduction pathway (by the agent capable of specifically interacting with p75). The effect of nerve regeneration by blocking of a signal transduction pathway has not been conventionally known. Therefore, the present invention
5 provides an effect more excellent than the prior art.

[0293]

In one embodiment of the present invention, the agent may be an agent capable of specifically interacting with a
10 polynucleotide encoding (a) a polynucleotide having the base sequence as set forth in SEQ ID NO. 3 or 16 or a fragment sequence thereof; (b) a polynucleotide encoding a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 4 or a fragment thereof; (c) a polynucleotide encoding a variant polypeptide
15 having the amino acid sequence as set forth in SEQ ID NO. 4 having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions and deletions, and having biological activity; (d) a polynucleotide which is a splice variant or allelic variant of the
20 base sequence as set forth in SEQ ID NO. 3 or 16; (e) a polynucleotide encoding a species homolog of the polypeptide having the amino acid sequence as set forth in SEQ ID NO. 4; (f) a polynucleotide hybridizable to any one of the polynucleotides described in (a) to (e) above under stringent
25 conditions and encoding a polypeptide having biological activity; or (g) a polynucleotide consisting of a base sequence having at least 70% identity to any one of the polynucleotides described in (a) to (e) or a complementary sequence thereof and encoding a polypeptide having biological activity.

30

[0294]

In one preferred embodiment, the number of substitutions, additions and deletions described in (c) above

may be limited to, for example, preferably 50 or less, 40 or less, 30 or less, 20 or less, 15 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. The number of substitutions, additions
5 and deletions is preferably small, but may be large as long as the biological activity is maintained (preferably, the activity is similar to or substantially the same as that of a product of the p75 gene).

10 [0295]

In another preferred embodiment, the biological activity possessed by the above-described variant polypeptide includes, but is not limited to, for example, an interaction with an antibody specific to the polypeptide having the amino
15 acid sequence as set forth in SEQ ID NO. 4 or a fragment thereof; an interaction with p75; modulation of the functional regulation of Rho GDI by p75; and the like. These activities can be measured by, for example, immunological assays, phosphorylation quantification, or the like.

20

[0296]

In another preferred embodiment, the allelic variant adventurously has at least 99% homology to the nucleic acid sequence as set forth in SEQ ID NO. 3 or 16.

25

[0297]

The above-described species homolog can be identified by searching a gene sequence database for the species of the species homolog using the p75 of the present invention as a
30 query sequence, if such a database is available. Alternatively, the species homolog can be identified by using the whole or part of p75 of the present invention as a probe or a primer to screen gene libraries of the species. Such an identification

method is well known in the art and is described in references as described herein. The species homolog preferably has at least about 30% homology to the nucleic acid sequence as set forth in SEQ ID NO: 3 or 16.

5

[0298]

In a preferred embodiment, the identity to any one of the polynucleotides described in (a) to (e) above or a complementary sequence thereof may be at least about 80%, more preferably at least about 90%, even more preferably at least about 98%, and most preferably at least about 99%.

[0299]

In a preferred embodiment, the nucleic acid molecule of the present invention encoding p75 or fragments and variants thereof may have a length of at least 8 contiguous nucleotides. The appropriate nucleotide length of the nucleic acid molecule of the present invention may vary depending on the purpose of use of the present invention. More preferably, the nucleic acid molecule of the present invention may have a length of at least 10 contiguous nucleotides, even more preferably at least 15 contiguous nucleotides, and still even more preferably at least 20 contiguous nucleotides. These lower limits of the nucleotide length may be present between the above-specified numbers (e. g., 9, 11, 12, 13, 14, 16, and the like) or above the above-specified numbers (e. g., 21, 22, ..., 30, and the like). The upper limit of the length of the nucleic acid molecule of the present invention may be greater than or equal to the full length of the sequence as set forth in SEQ ID NO. 3 or 16 as long as the polynucleotide can be used for the intended purpose (e. g. marker, primer and probe). Alternatively, when the nucleic acid molecule of the present invention is used as a primer, the nucleic acid molecule typically may have a

nucleotide length of at least about 8, preferably a nucleotide length of about 10. When used as a probe, the nucleic acid molecule typically may have a nucleotide length of at least about 15, and preferably a nucleotide length about 17.

5

[0300]

In one embodiment, the nucleic acid molecule encoding p75 or fragments or variants thereof comprise amino acids 114 to 1397 of the nucleic acid sequence as set forth in SEQ ID NO. 3 or amino acids 114 to 1397 of the nucleic acid sequence as set forth in SEQ ID NO. 16. More preferably, the nucleic acid molecule encoding p75 or fragments or variants thereof consist of amino acids 1 to 3386 of the nucleic acid sequence as set forth in SEQ ID NO. 3 or amino acids 1 to 3259 of the nucleic acid sequence as set forth in SEQ ID NO. 16.

[0301]

In one embodiment, nervous diseases, disorders or conditions to be treated include Alzheimer's disease, spinal cord injury, cerebrovascular disorder, brain injury, and the like. Preferably, a nervous disease, disorder or condition intended to be treated by the composition of the present invention may be Alzheimer's disease. In another preferred embodiment, nervous diseases, disorders or conditions intended to be treated by the composition of the present invention may be spinal cord injury, cerebrovascular disorder, and brain injury.

[0302]

In a preferred embodiment, the agent of the present invention is selected from the group consisting of a nucleic acid molecule, a polypeptide, a lipid, a sugar chain, a organic low molecule and a composite molecule thereof.

[0303]

In a preferred embodiment, the agent of the present invention is a nucleic acid molecule. When the agent of the present invention is a nucleic acid molecule, such a nucleic acid molecule may have a length of at least 8 contiguous nucleotides. The appropriate nucleotide length of the nucleic acid molecule of the present invention may vary depending on the purpose of use of the present invention. More preferably, the nucleic acid molecule of the present invention may have a length of at least 10 contiguous nucleotides, even more preferably at least 15 contiguous nucleotides, and still even more preferably at least 20 contiguous nucleotides. These lower limits of the nucleotide length may be present between the above-specified numbers (e. g., 9, 11, 12, 13, 14, 16, and the like) or above the above-specified numbers (e.g., 21, 22,...30, and the like). The upper limit of the length of the polynucleotide of the present invention may be greater than or equal to the full length of the sequence as set forth in SEQ ID NO: 3 or 16 as long as the polynucleotide can be used for the intended purpose (e. g. marker, primer and probe). Alternatively, when the nucleic acid molecule of the present invention is used as a primer, the nucleic acid molecule typically may have a nucleotide length of at least about 8, preferably a nucleotide length of about 10. When used as a probe, the nucleic acid molecule typically may have a nucleotide length of at least about 15, and preferably a nucleotide length about 17.

[0304]

Therefore, in an illustrative embodiment, the agent of the present invention may be a nucleic acid molecule sequence having a sequence complementary to any of the nucleic acid

sequences of the polynucleotides (a) to (g) or a sequence having at least 70% identity thereto.

[0305]

5 In another illustrative embodiment, the agent of the present invention may be a nucleic acid molecule hybridizable to any of the nucleic acid sequences of the polynucleotides (a) to (g).

10 [0306]

(p75 Extracellular Domain in the Polypeptide Form)

 In one aspect, the present invention provides a composition comprising a p75 extracellular domain polypeptide for regenerating nerves, and a composition comprising a p75
15 extracellular domain polypeptide for treatment, prophylaxis, diagnosis or prognosis of nervous diseases, nervous disorders or nervous conditions. An effective amount of the composition for regeneration, diagnosis, prophylaxis, treatment, or prognosis can be determined by those skilled in the art based
20 on the disclosures of the present specification using techniques well known in the art with reference to various parameters. For example, such an amount can be determined by those skilled in the art with reference to the purpose of use, a target disease (type, severity, and the like), the patient's
25 age, weight, sex and case history, the form or type of the cells, and the like (see Shinkei-Naika Chiryo Gaido [Guidance to Treatments in Neurological Internal Medicine], Norio Ogawa, Chugai-Igaku 1994). In the present invention, it was revealed that regeneration of nerves occurs due to inhibition of neurite
30 outgrowth being disrupted by blocking of the p75 signal transduction pathway (by the p75 extracellular domain polypeptide). The effect of nerve regeneration by blocking of a signal transduction pathway has not been conventionally

known. Therefore, the present invention provides an effect more excellent than the prior art.

[0307]

5 In one embodiment, the p75 extracellular domain of the present invention comprises (a) a polypeptide encoded by nucleotides 198 to 863 or nucleotides 201 to 866 of the nucleic acid sequence as set forth in SEQ ID NO. 3 or 16 or a fragment thereof; (b) a polypeptide having amino acids 29 to 250 or
10 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4 or a fragment thereof; (c) a variant polypeptide having amino acids 29 to 250 or 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4 having at least one mutation selected from the group consisting of one or more amino acid sub-
15 stitutions, additions and deletions, and having biological activity; (d) a polypeptide encoded by a splice variant or allelic variant of nucleotides 198 to 863 or 201 to 866 of the base sequence as set forth in SEQ ID NO. 3 or 16, respectively; (e) a polypeptide which is a species homolog
20 of a polypeptide having amino acids 29 to 250 or 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4, respectively; or (f) a polypeptide consisting of an amino acid sequence having at least 70% identity to any one of the polypeptides described in (a) to (e), and having biological
25 activity.

[0308]

 In one preferred embodiment, the number of substitutions, additions and deletions described in (b) above
30 may be limited to, for example, preferably 50 or less, 40 or less, 30 or less, 20 or less, 15 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. The number of substitutions, additions

and deletions is preferably smaller, but may be large as long as the biological activity is maintained (preferably, the activity is similar to or substantially the same as that of a product of the p75 gene).

5

[0309]

In another preferred embodiment, the allelic variant described in (c) above preferably has at least 99% homology to the amino acid sequence as set forth in SEQ ID NO. 4.

10

[0310]

In another preferred embodiment, the above-described species homolog can be identified as described above in the specification and preferably has at least about 30% homology to the amino acid sequence as set forth in SEQ ID NO. 4.

15

[0311]

The above-described species homolog can be identified by searching a gene sequence database for the species of the species homolog using the p75 of the present invention as a query sequence, if such a database is available. Alternatively, the species homolog can be identified by using the whole or part of p75 of the present invention as a probe or a primer to screen gene libraries of the species. Such an identification method is well known in the art and is described in references as described herein. The species homolog preferably has at least about 30% homology to the nucleic acid sequence as set forth in SEQ ID NO. 3 or 16 or the amino acid sequence as set forth in SEQ ID NO. 4.

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[0312]

In another preferred embodiment, the biological activity possessed by the variant polypeptide described in

(e) above includes, but is not limited to, for example, an interaction with an antibody specific to the polypeptide consisting of the amino acid sequence as set forth in SEQ ID NO. 4 or a fragment thereof; an interaction with the Pep5 polypeptide; an interaction with Rho, an interaction with GT1b, an interaction with MAG, an interaction with NgR, an interaction with Nogo, an interaction with OMgp, the modulation of the functional regulation of Rho GDI by p75; and the like. These interactions can be measured by immunoassays, phosphorylation quantification, and the like.

[0313]

In a preferred embodiment, the above-described homology to any one of the polypeptides described in (a) to (d) above may be at least about 80%, more preferably at least about 90%, even more preferably at least about 98%, and most preferably at least about 99%.

[0314]

The polypeptide of the present invention typically has a sequence of at least 3 contiguous amino acids. The amino acid length of the polypeptide of the present invention may be short as long as the peptide is suitable for an intended application, but preferably a longer sequence may be used. Therefore, the amino acid length may be preferably at least 4, more preferably at least 5, at least 6, at least 7, at least 8, at least 9 and at least 10, even more preferably at least 15, and still even more preferably at least 20. These lower limits of the amino acid length may be present between the above-specified numbers (e. g., 11, 12, 13, 14, 16, and the like) or above the above-specified numbers (e. g., 21, 22, ... 30, and the like). The upper limit of the length of the polypeptide of the present invention may be greater than or equal to the

full length of the sequence as set forth in SEQ ID NO. 4 as long as the peptide is capable of interacting with a given agent.

5 [0315]

In one embodiment, the p75 extracellular domain polypeptide or fragments or variants thereof comprise amino acids 29 to 250 or 30 to 251 of SEQ ID NO. 4 or 17, respectively. More preferably, the p75 extracellular domain polypeptide or
10 fragments or variants thereof consist of amino acids 29 to 250 or 30 to 251 of SEQ ID NO. 4 or 17, respectively.

[0316]

In one embodiment, nervous diseases, disorders or
15 conditions to be treated are exemplified herein elsewhere and include, for example, Alzheimer's disease, spinal cord injury, cerebrovascular disorder, brain injury, and the like. Preferably, a nervous disease, disorder or condition intended to be treated by the composition of the present invention may
20 be Alzheimer's disease. In another preferred embodiment, nervous diseases, disorders or conditions intended to be treated by the composition of the present invention may be spinal cord injury, cerebrovascular disorder, and brain injury.

25

[0317]

In another embodiment, the p75 extracellular domain of the present invention is preferably soluble. Such a soluble peptide can be prepared by removing the whole or a part of
30 the transmembrane domain using genetic engineering or synthesis.

[0318]

(p75 Extracellular Domain Polypeptide in the Nucleic Acid Form)

In one aspect, the present invention provides a composition comprising a nucleic acid molecule encoding the p75 extracellular domain polypeptide for regenerating nerves, and a composition comprising a nucleic acid molecule encoding the p75 extracellular domain polypeptide for treatment, prophylaxis, diagnosis or prognosis of nervous diseases, nervous disorders or nervous conditions. An effective amount of the composition for regeneration, diagnosis, prophylaxis, treatment, or prognosis can be determined by those skilled in the art based on the disclosures of the present specification using techniques well known in the art with reference to various parameters. For example, such an amount can be determined by those skilled in the art with reference to the purpose of use, a target disease (type, severity, and the like), the patient's age, weight, sex and case history, the form or type of the cells, and the like (see Shinkei-Naika Chiryo Gaido [Guidance to Treatments in Neurological Internal Medicine], Norio Ogawa, Chugai-Igaku 1994). In the present invention, it was revealed that regeneration of nerves occurs due to inhibition of neurite outgrowth being disrupted by blocking of the p75 signal transduction pathway (by the p75 extracellular domain). The effect of nerve regeneration by blocking of a signal transduction pathway has not been conventionally known. Therefore, the present invention provides an effect more excellent than the prior art.

[0319]

In one embodiment, the p75 extracellular domain of the present invention comprise a polynucleotide selected from the group consisting of (a) a polynucleotide having nucleotides 198 to 863 or nucleotides 201 to 866 of the base sequence as

set forth in SEQ ID NO. 3 or 16, respectively, or a fragment thereof; (b) a polynucleotide encoding amino acids 29 to 250 or 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4 or 17 or a fragment thereof; (c) a polynucleotide
5 encoding a variant polypeptide having amino acids 29 to 250 or 30 to 251 of the amino acid sequence as set forth in SEQ ID NO. 4 or 17 having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions and deletions, and having biological activity; (d)
10 a polynucleotide which is a splice variant or allelic variant of nucleotides 198 to 863 or 201 to 866 of the base sequence as set forth in SEQ ID NO. 3 or 16, respectively; (e) a polynucleotide encoding a species homolog of a polypeptide having amino acids 29 to 250 or 30 to 251 of the amino acid
15 sequence as set forth in SEQ ID NO. 4; (f) a polynucleotide hybridizable to any one of the polynucleotide described in (a) to (e), and encoding a polypeptide having biological activity; and (g) a polynucleotide consisting of a base sequence having at least 70% identity to any one of the
20 polynucleotides described in (a) to (e) or a complementary sequence thereof and encoding a polypeptide having biological activity.

[0320]

25 In one preferred embodiment, the number of substitutions, additions and deletions described in (c) above may be limited to, for example, preferably 50 or less, 40 or less, 30 or less, 20 or less, 15 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less,
30 3 or less, or 2 or less. The number of substitutions, additions and deletions is preferably smaller, but may be large as long as the biological activity is maintained (preferably, the activity is similar to or substantially the same as that of

a product of the p75 extracellular domain polypeptide).

[0321]

In another preferred embodiment, the biological
5 activity possessed by the above-described variant polypeptide
includes, but is not limited to, for example, an interaction
with an antibody specific to the polypeptide consisting of
the amino acid sequence as set forth in SEQ ID NO. 4 or a fragment
thereof; an interaction with an antibody specific to a
10 polypeptide consisting of an amino acid sequence as set forth
in SEQ ID NO. 4 or a fragment thereof; an interaction with
the Pep5; an interaction with Rho, an interaction with GT1b,
an interaction with MAG, an interaction with NgR, an interaction
with Nogo, an interaction with OMgp; modulation of the
15 functional regulation of Rho GDI by p75; and the like. These
activities can be measured by, for example, immunological
assays, phosphorylation quantification, or the like.

[0322]

20 In another preferred embodiment, the allelic variant
described above adventurously has at least 99% homology to
the nucleic acid sequence as set forth in SEQ ID NO. 3 or 16.

[0323]

25 The above-described species homolog can be identified
by searching a gene sequence database for the species of the
species homolog using the p75 extracellular domain of the
present invention as a query sequence, if such a database is
available. Alternatively, the species homolog can be
30 identified by using the whole or part of the p75 extracellular
domain of the present invention as a probe or a primer to screen
gene libraries of the species. Such an identification method
is well known in the art and is described in references as

described herein. For example, the species homolog preferably has at least about 30% homology to the nucleic acid sequence as set forth in SEQ ID NO. 3 or 16.

5 [0324]

In a preferred embodiment, the identity to any one of the polynucleotides described in (a) to (e) above or a complementary sequence thereof may be at least about 80%, more preferably at least about 90%, even more preferably at least about 98%, and most preferably at least about 99%.

[0325]

In a preferred embodiment, the nucleic acid molecule of the present invention encoding the p75 extracellular domain polypeptide or fragments and variants thereof may have a length of at least 8 contiguous nucleotides. The appropriate nucleotide length of the nucleic acid molecule of the present invention may vary depending on the purpose of use of the present invention. More preferably, the nucleic acid molecule of the present invention may have a length of at least 10 contiguous nucleotides, even more preferably at least 15 contiguous nucleotides, and still even more preferably at least 20 contiguous nucleotides. These lower limits of the nucleotide length may be present between the above-specified numbers (e.g., 9, 11, 12, 13, 14, 16, and the like) or above the above-specified numbers (e. g., 21, 22, ...30, and the like). The upper limit of the length of the polynucleotide of the present invention may be greater than or equal to the full length of the sequence as set forth in SEQ ID NO. 1 as long as the polynucleotide can be used for the intended purpose (e. g. marker, primer and probe). Alternatively, when the nucleic acid molecule of the present invention is used as a primer, the nucleic acid molecule typically may have a nucleotide length of at least

about 8, preferably a nucleotide length of about 10. When used as a probe, the nucleic acid molecule typically may have a nucleotide length of at least about 15, and preferably a nucleotide length about 17.

5

[0326]

In one embodiment, the nucleic acid molecule encoding the p75 extracellular domain polypeptide or fragments or variants thereof comprise nucleotides 198 to 863 or 201 to 866 of the nucleic acid sequence as set forth in SEQ ID NO. 3 or 16, respectively. More preferably, the nucleic acid molecule encoding the p75 extracellular domain or fragments or variants thereof consist of nucleotides 198 to 863 or 201 to 866 of the nucleic acid sequence as set forth in SEQ ID NO. 3 or 16, respectively.

[0327]

In one embodiment, nervous diseases, disorders or conditions to be treated are exemplified herein elsewhere and include, for example, Alzheimer's disease, spinal cord injury, cerebrovascular disorder, brain injury, and the like. Preferably, a nervous disease, disorder or condition intended to be treated by the composition of the present invention may be Alzheimer's disease. In another preferred embodiment, nervous diseases, disorders or conditions intended to be treated by the composition of the present invention may be spinal cord injury, cerebrovascular disorder, and brain injury.

30 [0328]

In another embodiment, the p75 extracellular domain polypeptide of the present invention is preferably soluble. Such a soluble peptide can be prepared by removing the whole

or a part of the transmembrane domain using genetic engineering or synthesis.

[0329]

5 (Agent Specifically Interacting with Rho GDI Polypeptide)

In one aspect, the present invention provides a composition comprising an agent capable of specifically interacting with a Rho GDI polypeptide for regenerating nerves, and a composition comprising an agent capable of specifically interacting with a Rho GDI polypeptide for treatment, prophylaxis, diagnosis or prognosis of nervous diseases, nervous disorders or nervous conditions. An effective amount of the composition for regeneration, diagnosis, prophylaxis, treatment, or prognosis can be determined by those skilled in the art based on the disclosures of the present specification using techniques well known in the art with reference to various parameters. For example, such an amount can be determined by those skilled in the art with reference to the purpose of use, a target disease (type, severity, and the like), the patient's age, weight, sex and case history, the form or type of the cells, and the like (see Shinkei-Naika Chiryo Gaido [Guidance to Treatments in Neurological Internal Medicine], Norio Ogawa, Chugai-Igaku 1994). In the present invention, it was revealed that regeneration of nerves occurs due to inhibition of neurite outgrowth being disrupted by blocking of the p75 signal transduction pathway (by the agent specifically interacting with the Rho GDI polypeptide). The effect of nerve regeneration by blocking of a signal transduction pathway has not been conventionally known. Therefore, the present invention provides an effect more excellent than the prior art.

[0330]

In one embodiment of the present invention, the agent of the present invention may be an agent capable of specifically interacting with (a) a polypeptide encoded by the nucleic acid sequence as set forth in SEQ ID NO. 5 or a fragment thereof; 5 (b) a polypeptide having an amino acid sequence as set forth in SEQ ID NO. 6; (c) a variant polypeptide having an amino acid sequence as set forth in SEQ ID NO. 6 having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions and deletions, and having 10 biological activity; (d) a polypeptide encoded by a splice variant or allelic variant of the base sequence as set forth in SEQ ID NO. 5; (e) a polypeptide which is a species homolog of the amino having an acid sequence as set forth in SEQ ID NO. 6; or (f) a polypeptide having an amino acid sequence having 15 at least 70% identity to any one of the polypeptides described in (a) to (e), and having biological activity.

[0331]

In one preferred embodiment, the number of substitutions, additions and deletions described in (b) above 20 may be limited to, for example, preferably 50 or less, 40 or less, 30 or less, 20 or less, 15 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. The number of substitutions, additions 25 and deletions is preferably smaller, but may be large as long as the biological activity is maintained (preferably, the activity is similar to or substantially the same as that of a product of the Rho GDI gene).

30 [0332]

In a preferred embodiment, the agent of the present invention is selected from the group consisting of a nucleic acid molecule, a polypeptide, a lipid, a sugar chain, an organic

low molecule, and a composite molecule thereof.

[0333]

5 In another preferred embodiment, the allelic variant described in (c) above preferably has at least 99% homology to the amino acid sequence as set forth in SEQ ID NO. 4.

[0334]

10 In another preferred embodiment, the above-described species homolog can be identified as described above and preferably has at least about 30% homology to the amino acid sequence as set forth in SEQ ID NO. 6.

[0335]

15 In another preferred embodiment, the biological activity possessed by the variant polypeptide described in (e) above includes, but not limited to, for example, an interaction with an antibody specific to the polypeptide having the amino acid sequence as set forth in SEQ ID NO. 6 or a fragment thereof; an interaction with the p75 polypeptide; and the like.

[0336]

25 In a preferred embodiment, the above-described homology to any one of the polypeptides described in (a) to (d) above may be at least about 80%, more preferably at least about 90%, even more preferably at least about 98%, and most preferably at least about 99%.

[0337]

30 The polypeptide with which the agent of the present invention specifically interacts typically has a sequence of at least 3 contiguous amino acids. The amino acid length of the polypeptide of the present invention may be short as long

as the polynucleotide is suitable for an intended application, but preferably a longer sequence may be used. Therefore, the amino acid length may be preferably at least 4, more preferably at least 5, at least 6, at least 7, at least 8, at least 9 and at least 10, even more preferably at least 15, and still even more preferably at least 20. These lower limits of the amino acid length may be present between the above-specified numbers (e.g., 11, 12, 13, 14, 16, and the like) or above the above-specified numbers (e.g., 21, 22, ...30, and the like). The upper limit of the length of the polypeptide of the present invention may be greater than or equal to the full length of the sequence as set forth in SEQ ID NO. 6 as long as the peptide is capable of interacting with a given agent.

[0338]

In a preferred embodiment, the agent of the present invention is selected from the group consisting of a nucleic acid molecule, a polypeptide, a lipid, a sugar chain, an organic low molecule and a composite molecule thereof. More preferably, the agent of the present invention is antibody or a derivative thereof (e. g., a single chain antibody). Therefore, the agent of the present invention can be used as a probe.

[0339]

In one embodiment, the Rho GDI polypeptide or fragments or variants thereof comprise the whole amino acid sequence as set forth in SEQ ID NO. 6. More preferably, the Rho GDI or fragments or variants thereof consist of the whole amino acid sequence as set forth in SEQ ID NO. 6.

[0340]

In one embodiment, nervous diseases, disorders or conditions to be treated are exemplified herein elsewhere and

include, for example, Alzheimer's disease, spinal cord injury, cerebrovascular disorder, brain injury, and the like. Preferably, a nervous disease, disorder or condition intended to be treated by the composition of the present invention may
5 be Alzheimer's disease. In another preferred embodiment, nervous diseases, disorders or conditions intended to be treated by the composition of the present invention may be spinal cord injury, cerebrovascular disorder, and brain
10 injury.

[0341]

(Agent Interacting with a Nucleic Acid Molecule Encoding the Rho GDI Polypeptide)

In one aspect, the present invention provides a
15 composition comprising an agent specifically interacting with a nucleic acid molecule encoding the Rho GDI polypeptide for regenerating nerves, and a composition comprising an agent of specifically interacting with a nucleic acid molecule encoding the Rho GDI polypeptide for treatment, prophylaxis,
20 diagnosis or prognosis of nervous diseases, nervous disorders or nervous conditions. An effective amount of the composition for regeneration, diagnosis, prophylaxis, treatment, or prognosis can be determined by those skilled in the art based on the disclosures of the present specification using
25 techniques well known in the art with reference to various parameters. For example, such an amount can be determined by those skilled in the art with reference to the purpose of use, a target disease (type, severity, and the like), the patient's age, weight, sex and case history, the form or type of the
30 cells, and the like (see Shinkei-Naika Chiryo Gaido [Guidance to Treatments in Neurological Internal Medicine], Norio Ogawa, Chugai-Igaku 1994). In the present invention, it was revealed that regeneration of nerves occurs due to inhibition of neurite

outgrowth being disrupted by blocking of the p75 signal transduction pathway (by the agent capable of specifically interacting with the Rho GDI polypeptide). The effect of nerve regeneration by blocking of a signal transduction pathway has not been conventionally known. Therefore, the present invention provides an effect more excellent than the prior art.

[0342]

10 In one embodiment of the present invention, the agent may be an agent specifically interacting with (a) a polynucleotide having the base sequence as set forth in SEQ ID NO. 5 or a fragment thereof; (b) a polynucleotide encoding a polypeptide having an amino acid sequence as set forth in
15 SEQ ID NO. 6 or a fragment thereof; (c) a polynucleotide encoding a variant polypeptide having the amino acid sequence as set forth in SEQ ID NO. 6 having at least one mutation selected from the group consisting of one or more amino acid substitutions, additions and deletions, and having biological
20 activity; (d) a polynucleotide which is a splice variant or allelic variant of the base sequence as set forth in SEQ ID NO. 5; (e) a polynucleotide encoding a species homolog of the polypeptide consisting of the amino acid sequence as set forth in SEQ ID NO. 6; (f) a polynucleotide hybridizable to any one
25 of the polynucleotides described in (a) to (e) above under stringent conditions and encoding a polypeptide having biological activity; or (g) a polynucleotide having a base sequence consisting of at least 70% identity to any one of the polynucleotides described in (a) to (e) or a complementary
30 sequence thereof and encoding a polypeptide having biological activity.

[0343]

In one preferred embodiment, the number of substitutions, additions and deletions described in (c) above may be limited to, for example, preferably 50 or less, 40 or less, 30 or less, 20 or less, 15 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. The number of substitutions, additions and deletions is preferably smaller, but may be large as long as the biological activity is maintained (preferably, the activity is similar to or substantially the same as that of a product of the Rho GDI gene).

[0344]

In another preferred embodiment, the biological activity possessed by the above-described variant polypeptide includes, but is not limited to, for example, an interaction with an antibody specific to the polypeptide having the amino acid sequence as set forth in SEQ ID NO. 6 or a fragment thereof; an interaction with p75; modulation of the functional regulation of Rho GDI by p75; and the like. These activities can be measured by, for example, immunological assays, phosphorylation quantification, or the like.

[0345]

In another preferred embodiment, the allelic variant described above has at least 99% homology to the nucleic acid sequence as set forth in SEQ ID NO. 5.

[0346]

The above-described species homolog can be identified by searching a gene sequence database for the species of the species homolog using the Rho GDI of the present invention as a query sequence, if such a database is available. Alternatively, the species homolog can be identified by using

the whole or part of the Rho GDI of the present invention as a probe or a primer to screen gene libraries of the species. Such an identification method is well known in the art and is described in references as described herein. For example,
5 the species homolog preferably has at least about 30% homology to the nucleic acid sequence as set forth in SEQ ID NO. 5.

[0347]

10 In a preferred embodiment, the identity to any one of the polynucleotides described in (a) to (e) above or a complementary sequence thereof may be at least about 80%, more preferably at least about 90%, even more preferably at least about 98%, and most preferably at least about 99%.

15 [0348]

In a preferred embodiment, the nucleic acid molecule of the present invention encoding Rho GDI or fragments and variants thereof may have a length of at least 8 contiguous nucleotides. The appropriate nucleotide length of the nucleic
20 acid molecule of the present invention may vary depending on the purpose of use of the present invention. More preferably, the nucleic acid molecule of the present invention may have a length of at least 10 contiguous nucleotides, even more preferably at least 15 contiguous nucleotides, and still even
25 more preferably at least 20 contiguous nucleotides. These lower limits of the nucleotide length may be present between the above-specified numbers (e. g., 9, 11, 12, 13, 14, 16, and the like) or above the above-specified numbers (e. g., 21, 22,...30, and the like). The upper limit of the length of
30 the polynucleotide of the present invention may be greater than or equal to the full length of the sequence as set forth in SEQ ID NO. 5 as long as the polynucleotide can be used for the intended purpose (e. g. marker, primer, probe). Al-

ternatively, when the nucleic acid molecule of the present invention is used as a primer, the nucleic acid molecule typically may have a nucleotide length of at least about 8, preferably a nucleotide length of about 10. When used as a probe, the nucleic acid molecule typically may have a nucleotide length of at least about 15, and preferably a nucleotide length about 17.

[0349]

10 In one embodiment, the nucleic acid molecule encoding the Rho GDI polypeptide or fragments or variants thereof comprise the whole amino acid sequence as set forth in SEQ ID NO. 5. More preferably, the nucleic acid molecule encoding the Rho GDI or fragments or variants thereof consists of the whole amino acid sequence as set forth in SEQ ID NO. 5.

[0350]

20 In one embodiment, nervous diseases, disorders or conditions to be treated are exemplified herein elsewhere and include, for example, Alzheimer's disease, spinal cord injury, cerebrovascular disorder, brain injury, and the like. Preferably, a nervous disease, disorder or condition intended to be treated by the composition of the present invention may be Alzheimer's disease. In another preferred embodiment, 25 nervous diseases, disorders or conditions intended to be treated by the composition of the present invention may be spinal cord injury, cerebrovascular disorder, and brain injury.

30 [0351]

In a preferred embodiment, the agent of the present invention is selected from the group consisting of a nucleic acid molecule, a polypeptide, a lipid, a sugar chain, an organic

low molecule and a composite molecule thereof.

[0352]

In a preferred embodiment, the agent of the present invention is a nucleic acid molecule. When the agent of the present invention is a nucleic acid molecule, such a nucleic acid molecule may have a length of at least 8 contiguous nucleotides. The appropriate nucleotide length of the nucleic acid molecule of the present invention may vary depending on the purpose of use of the present invention. More preferably, the nucleic acid molecule of the present invention may have a length of at least 10 contiguous nucleotides, even more preferably at least 15 contiguous nucleotides, and still even more preferably at least 20 contiguous nucleotides. These lower limits of the nucleotide length may be present between the above-specified numbers (e. g., 9, 11, 12, 13, 14, 16, and the like) or above the above-specified numbers (e. g., 21, 22, ...30, and the like). The upper limit of the length of the polynucleotide of the present invention may be greater than or equal to the full length of the sequence as set forth in SEQ ID NO. 5 as long as the polynucleotide can be used for the intended purpose (e. g., marker, primer and probe). Alternatively, when the nucleic acid molecule of the present invention is used as a primer, the nucleic acid molecule typically may have a nucleotide length of at least about 8, preferably a nucleotide length of about 10. When used as a probe, the nucleic acid molecule typically may have a nucleotide length of at least about 15, and preferably a nucleotide length about 17.

30

[0353]

Therefore, in an illustrative embodiment, the agent of the present invention may be a nucleic acid molecule sequence

having a sequence complementary to any of the nucleic acid sequences of the polynucleotides (a) to (g) or a sequence having at least 70% identity thereto.

5 [0354]

In another illustrative embodiment, the agent of the present invention may be a nucleic acid molecule hybridizable to any of the nucleic acid sequences of the polynucleotides (a) to (g) under stringent conditions.

10

[0355]

In another preferred embodiment, therefore, the present invention provides a composition for disrupting neurite outgrowth inhibition.

15

[0356]

(Method for Nerve Regeneration)

In another aspect, the present invention provides a method for regenerating nerves. This method comprises the step of providing a composition comprising at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, Rho GDI polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide to the nerve in an amount effective for regeneration. In the nerve regeneration method of the present invention, an amount effective for nerve regeneration can be determined by those skilled in the art using techniques well known in the art with reference to various parameters, including the purpose of use,

a target disease (type, severity, and the like), the patient's age, weight, sex and case history, the form or type of the cells, and the like (see Shinkei-Naika Chiryō Gaido [Guidance to Treatments in Neurological Internal Medicine], Norio Ogawa, Chugai-Igaku 1994). In the present invention, it was revealed that regeneration of nerves occurs due to inhibition of neurite outgrowth being disrupted by blocking of the p75 signal transduction pathway (e. g., via an agent related to the p75 signal transduction pathway). The effect of nerve regeneration by blocking of the signal transduction pathway has not been conventionally known. Therefore, the present invention provides an effect more excellent than the conventional art.

[0357]

In one embodiment, the Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent capable of specifically interacting with the p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, the p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, Rho GDI polypeptide and a nucleic acid molecule encoding the Rho GDI polypeptide can be in forms as described above in the specification. In the present invention, it was revealed that regeneration of nerves occurs due to inhibition of neurite outgrowth being disrupted by blocking of the p75 signal transduction pathway. The effect of nerve regeneration by blocking of the signal transduction pathway has not been conventionally known. Therefore, the present invention provides an effect more excellent than the conventional art. Particularly, a plurality of the Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with the p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding

the p75 polypeptide, the p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, the Rho GDI polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide may be preferably used. In such a case, various combinations may be used. Preferably, two, three or four polypeptides, polynucleotides and/or agents may be used. In another preferred embodiment, a plurality of molecules may be advantageously inhibited on the pathway.

[0358]

In another aspect, the present invention provides a composition for regenerating nerves, comprising a plurality of elements of the Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent capable of specifically interacting with the p75 polypeptide, an agent capable of specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, the p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, the Rho GDI polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide. In this case, various combinations may be used. Preferably, two, three or four polypeptides, polynucleotides and/or agents can be used. In another preferred embodiment, a substance inhibiting a plurality of molecules on the pathway may be advantageously used.

[0359]

(Diagnosis, Prophylaxis, Treatment or Prognosis for Neurological Diseases, Disorders or Conditions)

In another aspect, the present invention provides a method for diagnosis, prophylaxis, treatment or prognosis for neurological diseases, disorders or conditions. This method

comprises a step of providing a composition comprising at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, Rho GDI polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide to the nerve in an amount effective for regeneration. An amount effective for nerve regeneration can be determined by those skilled in the art using techniques well known in the art with reference to various parameters, including the purpose of use, a target disease (type, severity, and the like), the patient's age, weight, sex and case history, the form or type of the cells, and the like (see Shinkei-Naika Chiryo Gaido [Guidance to Treatments in Neurological Internal Medicine], Norio Ogawa, Chugai-Igaku 1994). In the present invention, it was revealed that regeneration of nerves occurs due to inhibition of neurite outgrowth being disrupted by blocking of the p75 signal transduction pathway (e. g., via an agent related to the p75 signal transduction pathway). The effect of nerve regeneration by blocking of the signal transduction pathway has not been conventionally known. Therefore, the present invention provides an effect more excellent than the conventional art.

[0360]

In one embodiment, the Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with the p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, the p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain

polypeptide, the Rho GDI polypeptide and a nucleic acid molecule encoding the Rho GDI polypeptide can be in forms as described above in the specification. In the present invention, it was revealed that regeneration of nerves occurs
5 due to inhibition of neurite outgrowth being disrupted by blocking of the p75 signal transduction pathway. The effect of nerve regeneration by blocking of the signal transduction pathway has not been conventionally known. Therefore, the present invention provides an effect more excellent than the
10 conventional art. Particularly, a plurality of the Peps polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with the p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, the p75 ex-
15 tracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, the Rho GDI polypeptide and a nucleic acid molecule encoding the Rho GDI polypeptide may be preferably used. In such case, various combinations may be used. Preferably, two, three or four
20 polypeptides, polynucleotides and/or agents can be used. In another preferred embodiment, a plurality of molecules may be advantageously inhibited on the pathway.

[0361]

25 In another aspect, the present invention provides a composition for diagnosis, prophylaxis, treatment or prognosis for neurological diseases, disorders or conditions. This composition comprises at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid
30 molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic

acid molecule encoding the p75 extracellular domain polypeptide, Rho GDI polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide. Here, an amount effective for diagnosis, prophylaxis, treatment or prognosis can be
5 determined by those skilled in the art using techniques well known in the art with reference to various parameters, including the purpose of use, a target disease (type, severity, and the like), the patient's age, weight, sex and case history, the form or type of the cells, and the like.

10

[0362]

In another aspect, the present invention provides a composition for diagnosis, prophylaxis, treatment or prognosis for neurological diseases, disorders or conditions,
15 comprising a plurality of elements of the Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent capable of specifically interacting with the p75 polypeptide, an agent capable of specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, the p75 ex-
20 tracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, the Rho GDI polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide. In this case, various combinations may be used. Preferably, two, three or four polypeptides,
25 polynucleotides and/or agents can be used. In another preferred embodiment, a substance inhibiting a plurality of molecules on the pathway may be advantageously used.

[0363]

30

(Construction of a Network of Neurons)

In another aspect, the present invention also provides a composition for constructing a network of neurons.

[0364]

Here, construction of a network of neurons refers to interconnection between a plurality of neurons so that organic matter or information is transferred between the cells.

5 Neurons forming such a network are also referred to as a neuron population. Examples of neurons forming such a network include, but are not limited to, a population of neurons forming synapses, the brain, the spinal cord, the peripheral nerve, and the like.

10

[0365]

The composition for constructing a network of neurons comprises at least one molecule selected from the group consisting of a Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, Rho GDI polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide. Here, an amount effective for construction of a network of neurons can be determined by those skilled in the art using techniques well known in the art with reference to various parameters, including the purpose of use, a target disease (type, severity, and the like), the patient's age, weight, sex and case history, the form or type of the cells, and the like. In the present invention, it was revealed that regeneration of nerves occurs due to inhibition of neurite outgrowth being disrupted by blocking of the p75 signal transduction pathway. The effect of nerve regeneration by blocking of the signal transduction pathway has not been conventionally known. Therefore, the present invention provides an effect more excellent than the

30

conventional art.

[0366]

5 The thus-obtained neurons (population) forming a network can be transplanted to organisms having a nervous disorder.

[0367]

10 In one embodiment, the Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent capable of specifically interacting with the p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, the p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain
15 polypeptide, Rho GDI polypeptide and a nucleic acid molecule encoding the Rho GDI polypeptide can be in forms as described above in the specification. In the present invention, it was revealed that regeneration of nerves occurs due to inhibition of neurite outgrowth being disrupted by blocking of the p75
20 signal transduction pathway. The effect of nerve regeneration by blocking of the signal transduction pathway has not been conventionally known. Therefore, the present invention provides an effect more excellent than the conventional art. Particularly, a plurality of the Pep5 polypeptide, a nucleic
25 acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with the p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, the p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain
30 polypeptide, the Rho GDI polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide may be preferably used. In such a case, various combinations may be used. Preferably, two, three or four polypeptides, polynucleotides

and/or agents may be used. In another preferred embodiment, a plurality of molecules may be advantageously inhibited on the pathway.

5 [0368]

In another aspect, the present invention provides a method for constructing a network of neurons. This method comprises the step of providing a composition comprising at least one molecule selected from the group consisting of a
10 Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with a p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, a p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75
15 extracellular domain polypeptide, Rho GDI polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide to the neurons in an amount effective for construction of the network of the neurons.

20 [0369]

(Kit for Treatment of Nervous Diseases)

In another aspect, the present invention provides a kit for treatment of neurological diseases. This kit comprises (A) a population of cells regenerated using a composition
25 comprising at least one molecule selected from the group consisting of the Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with the p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75
30 polypeptide, the p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, the Rho GDI polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide, and (B) a container

for preserving the cell population.

[0370]

Alternatively, such a kit comprises: (A) a composition
5 comprising at least one molecule selected from the group
consisting of the Peps polypeptide, a nucleic acid molecule
encoding the Pep5 polypeptide, an agent specifically in-
teracting with the p75 polypeptide, an agent specifically
interacting with a nucleic acid molecule encoding the p75
10 polypeptide, the p75 extracellular domain polypeptide, a
nucleic acid molecule encoding the p75 extracellular domain
polypeptide, the Rho GDI polypeptide, and a nucleic acid
molecule encoding the Rho GDI polypeptide; (B) cells capable
of differentiating into neurons, and (C) a container for
15 preserving the cell population.

[0371]

The above kit is effective for treatment of diseases
(nervous diseases, nervous disorders, nervous abnormal
20 conditions, and the like) which require neurons or a neuron
population. The obtained neurons or neuron population may
be in any condition, but preferably, a differentiation
condition is suitable.

25 [0372]

Instructions provided in the kit of the present
invention may be in any form as long as the instruction can
be conveyable, including paper, computer readable recording
media (e. g., a flexible disk, CD-R, and the like), electric
30 mail, web sites, and the like.

[0373]

In another aspect, the present invention provides a

method for treatment of neurological diseases. This method comprise the steps of (a) providing a cell population regenerated with a composition comprising at least one molecule selected from the group consisting of the Pep5 polypeptide, a nucleic acid molecule encoding the Pep5 polypeptide, an agent specifically interacting with the p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, the p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, the Rho GDI polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide; and (b) transplanting the cell population to a patient.

[0374]

Such a cell population is also referred to as a graft. As used herein, the term "graft" typically refers to homologous or exogenous tissue or cells to be inserted into a specific site of the body, which serve as a part of the body after insertion. Examples of conventional grafts include organs or part of the organ, blood vessel, blood vessel-like tissue, skin segments, cardiac valve, pericardium, dura, cornea segments, teeth, and the like. Therefore, the graft encompasses any material used for compensating an impaired portion by inserting into the portion. Examples of the graft include, but are not limited to, autograft, allograft, and heterograft, depending on the type of the donor. As used herein, the term "immune reaction" refers to a reaction due to lack of coordination of immunological tolerance between a graft and a host, including, for example, hyperacute rejection (within several minutes immediately after transplant) (immune reaction due to β -Gal antibody or the like), acute rejection (reaction due to cell-mediated immunity 7 to 21 days after transplant), chronic rejection (rejection due to

cell-mediated immunity after three months or more), and the like. Whether or not an immune reaction is elicited can be herein determined by histopathologically studying the type or number of cells (immune system) infiltrating into graft
5 tissue by staining such as HE staining or the like, immunostaining, or microscopic examination of tissue sections.

[0375]

The provision of a cell population is described in detail
10 in other portions in the specification. For transplant of cells into a patient, techniques well known in the art can be used. Such techniques are described in Hyojun-Gekagaku [Standard Surgery] (published by Igakushoin), Shin-Gekagaku-Taikai (New Complete Surgery (published by
15 Nakayama-shoten), and the like. Preferably, when a graft of the present invention is transplanted, it may be preferably noted that an excessive pressure should be avoided in the above-described general methods.

20 [0376]

The graft or cell population of the present invention may comprise an immunosuppressant therein or therewith. Such an immunosuppressant is known in the art. For the purpose of immunosuppression, other methods for achieving immu-
25 nosuppression may be used. Examples of immunosuppression methods for avoiding the above-described rejection include use of an immunosuppressant, surgical operations, radiation exposure, and the like. Major immunosuppressants include an adrenocortical steroid drug, cyclosporine, FK506, and the like.
30 The adrenocortical steroid drug reduces the number of circulating T cells and inhibits the nucleic acid metabolism and cytokine secretion of lymphocytes to suppress the functions thereof and the migration and metabolism of macrophages. As

a result, an immune reaction can be suppressed. Cyclosporine and FK506 have similar functions in which they bind to a receptor present on the membrane of helper T cells and enter cells, and then directly act on DNA to inhibit production of interleukin-2. Killer T cells eventually cannot function, resulting in immunosuppression. Side effects are a problem with use of these immunosuppressants. Particularly, steroids cause a number of side effects and cyclosporine is toxic to the liver and the kidney. FK506 is also toxic to the kidney. Examples of a surgical operation include, for example, lymphnodectomy, splenectomy, and thymectomy, but the effect thereof has not been fully demonstrated. Among the surgical operations, thoracic duct funnel draws circulating lymphocytes to the outside of the body and its effectiveness has been confirmed, but it has a drawback such that a large volume of serum protein and lipid flow out nutritional disorder is likely to occur. Radiation exposure includes whole body radiation and graft radiation. The effect of radiation exposure is not reliable and the load of a recipient is large. Therefore, radiation exposure is used in combination with the above-described immunosuppressant. Any of the above-described methods is not very preferable for prevention of rejection.

[0377]

(Screening)

The present invention also provides a screening method for identifying an agent inducing nerve regeneration. In this method, such an agent can be identified by determining whether or not the test agent has a significant effect (reduction, enhancement, extinction, or the like) on the interaction between at least one molecule selected from the group consisting of the Pep5 polypeptide, a nucleic acid molecule

encoding the Pep5 polypeptide, an agent specifically interacting with the p75 polypeptide, an agent specifically interacting with a nucleic acid molecule encoding the p75 polypeptide, the p75 extracellular domain polypeptide, a nucleic acid molecule encoding the p75 extracellular domain polypeptide, the Rho GDI polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide, and molecules interacting therewith.

10 [0378]

In one embodiment, the method comprises the steps of (a) contacting a first polypeptide having an amino acid sequence having at least 70% homology to SEQ ID NO. 4 or a fragment thereof and a second polypeptide having an amino acid sequence having at least 70% homology to SEQ ID NO. 6 or a fragment thereof in the presence of a test agent, and (b) comparing the binding level of the first polypeptide and the second polypeptide in the presence of the test agent with the binding level thereof in the absence of the test agent, where when the binding level is reduced in the presence of the test agent as compared to when the test agent is absent, the test agent is identified as an agent for nerve regeneration.

[0379]

25 The above-described method for determining a test agent is well known in the art and the results can be analyzed using any statistical technique.

[0380]

30 In the identification method of the present invention, presentation and selection of subjects or patients can be arbitrarily carried out. However, in the case of human subjects, it is preferable to previously obtain the consent

of a human patient. Any subject having an abnormal nervous condition can be used.

[0381]

5 In an administration step in the identification method of the present invention, any technique may be used. Preferably, a form of administration used in ordinary therapies, such as oral administration, intravenous injection, or the like, is advantageous.

10

[0382]

 The above-described screening or identification method is well known in the art. The screening or identification method can be carried out using a microtiter plate or a biomolecule array or chip having DNA, protein, or the like. An agent to
15 be tested by screening may be contained in, for example, gene libraries, compound libraries synthesized by combinatorial libraries, and the like. However, the present invention is not limited thereto.

20

[0383]

 Therefore, the present invention is intended to provide a drug by computer modeling based on the disclosures of the present invention.

25

[0384]

 In other embodiments, the present invention includes compounds obtained by a quantitative structure activity relationship (QSAR) computer modeling technique as an
30 instrument for screening for the regulatory activity of the compound of the present invention. Here, the computer technique includes some substrate templates prepared by a computer, pharmacophore, production of homologous models of

the active site of the present invention, and the like. In general, a method for modeling an ordinary characteristic group of a substance capable of interacting with a given substance from data obtained in vitro can be carried out using a CATALYST™ pharmacophore method (Ekins et al., Pharmacogenetics, 9:477-489, 1999; Ekins et al., J. Pharmacol. & Exp. Ther., 288:21-29, 1999; Ekins et al., J. Pharmacol. & Exp. Ther., 290:429-438, 1999; Ekins et al., J. Pharmacol. & Exp. Ther., 291:424-433, 1999) and comparative molecular field analysis; COMFA) (Jones et al., Drug Metabolism & Disposition, 24:1-6, 1996), and the like. In the present invention, the computer modeling may be carried out using molecular modeling software (e. g., CATALYST™ version 4 (Molecular Simulations, Inc., San Diego, CA.), or the like).

[0385]

Fitting of a compound to an active site can be carried out using any computer modeling technique known in the art. Visual inspection and manual operation of a compound to an active site can be carried out using a program, such as QUANTA (Molecular Simulations, Burlington, Mass., 1992), SYBYL (Molecular Modeling Software, Tripos Associates, Inc., St. Louis, Mo., 1992), AMBER (Weiner et al., J. Am. Chem. Soc., 106:765-784, 1984), CHARMM (Brooks et al., J. Comp. Chem., 4:187-217, 1983), or the like. In addition, energy minimization can be carried out using a standard force field, such as CHARMM, AMBER, or the like. Other more specialized computer modelings include GRID (Goodford et al., J. Med. Chem., 28:849-857, 1985), MCSS (Miranker and Karplus, Function and Genetics, 11:29-34, 1991), AUTODOCK (Goodsell and Olsen, Proteins: Structure, Function and Genetics, 8:195-202, 1990), DOCK (Kuntz et al., J. Mol. Biol., 161:269-288, (1982)), and the like. Additional structures of compounds can be newly constructed to blank

active sites, active sites of known low molecular weight compounds, or the like, using a computer program, such as LUDI (Bohm, J. Comp. Aid. Molec. Design, 6:61-78, 1992), LEGEND (Nishibata and Itai, Tetrahedron, 47:8985, 1991), LeapFrog (Tripos Associates, St.Louis, Mo.), or the like. Such computer modelings are well known in the art and commonly used. Those skilled in the art can appropriately design compounds within the scope of the present invention in accordance with the disclosures of the present specification.

[0386]

In another aspect, the present invention provides a modulating agent which is identified by the above-described identification method of the present invention.

[0387]

In another aspect, the present invention provides a pharmaceutical composition comprising the modulating agent of the present invention.

[0388]

In another aspect, the present invention provides a method for prophylaxis or treatment of neurological diseases, disorders or conditions. Here, this method comprises the step of administering a pharmaceutical composition comprising the modulating agent of the present invention to a subject. Preferably, the nerve-related conditions, disorders or diseases include, but are not limited to, abnormalities, disorders or diseases for which the present invention is determined to be effective, preferably Alzheimer's disease.

[0389]

Nerve-related diseases, disorders and conditions have

been believed to be difficult to cure completely. However, the above-described effect of the present invention allows early diagnosis which has been conventionally believed to be impossible, and is applicable to therapies. Therefore, the present invention can be regarded to have usefulness which cannot be achieved by conventional diagnostics or medicaments.

[0390]

(Transgenic Animals)

In another aspect, the present invention also provides a vector comprising a nucleic acid molecule having the sequence of at least one nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding the Pep5 polypeptide, a nucleic acid molecule encoding the p75 polypeptide, and a nucleic acid molecule encoding the Rho GDI polypeptide. This vector can be used for various purposes, including, but limited to, production of transgenic animals, production of modified polypeptides, and the like.

[0391]

Therefore, the present invention provides a cell, tissue, an organ, and an organism comprising the above-described vector. The present invention also provides a nerve-modified transgenic animal transformed using the vector. A method for producing an animal is known in the art.

[0392]

In another aspect, the present invention provides a knockout animal in which a gene of the present invention is knocked out.

[0393]

As used herein, the term "knock out" with reference

to a gene refers to disruption (loss) or malfunctioning of the gene.

[0394]

5 As used herein, the term "knockout animal" refers to an animal (e. g., mouse) in which a given gene is knocked out.

[0395]

10 Any "animal" capable of being knocked out may be herein used as long as it can be knocked out. __Therefore, an animal encompasses a vertebrate and an invertebrate. An animal includes a mammal (e. g., mouse, dog, cat, rat, monkey, pig, cattle, sheep, rabbit, dolphin, whale, goat, horse, and the like), a bird (e. g., chicken, quail, and the like), an
15 amphibian (e.g., frog, etc.), a reptile, an insect (e. g., Drosophila, and the like), and the like. Preferably, an animal may be a mammal, more preferably an animal which is easy to knock out (e. g., mouse). In another preferred embodiment, an animal may be one that has been revealed to
20 be appropriate as a model animal for humans (e. g., monkey). In some embodiments, an animal may not be a human. However, the present invention is not limited thereto.

[0396]

25 Hereinafter, the present invention will be described based on examples, but the following examples are provided only by way of example. Therefore, the scope of the present invention is limited only by the accompanying claims but not the examples.

30

[0397]

[Examples]

 The present invention will be described in greater

detail below with reference to examples. The present invention is not limited to the examples below. The handling of animals complied with provisions defined by Osaka University.

5

[0398]

(Materials and Methods)

(Animals)

10 A strain of mice bearing a targeted disruption of the third exon of the p75^{NTR} gene (Cited Reference 23) was used. This mouse strain was originally obtained from the Jackson Laboratory (Bar Harbor, Maine) on a C57BL/6J background.

[0399]

15 (Co-immunoprecipitation)

Amino-terminally FLAG-tagged human p75^{NTR} (SEQ ID NOs. 3 and 4) and/or HA-tagged RhoA² (SEQ ID. NOs. 5 and 6) were transfected with 293T cells or N1E-115 cells by lipofection using Lipofectamine 2000 (Gibco BRL). Cells were lysed on
20 ice for 20 min with lysis buffer (10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.2% NP-40, 25 µg/ml leupeptin and 25 µg/ml aprotinin). The lysates were centrifuged at 13,000g for 20 min, and the supernatants were collected. They were then incubated with the anti-FLAG antibody (for transfected
25 FLAG-p75^{NTR}) or anti-p75 antibody (Chemicon) (for cerebellar neurons) for 3 hours. The immunocomplex was collected with protein A sepharose (Amersham Pharmacia). The suspension was centrifuged at 1,000g for 5 min. The pellets were washed 4 times with lysis buffer, and subjected to SDS-PAGE, followed
30 by immunoblot analysis using anti-Rho GDIα antibody (Sigma) or anti-RhoA antibody (Santa Cruz Biotechnology). Where indicated, recombinant rat MAG-Fc chimera (25 µg/ml, RD Systems Inc.), the Nogo peptide (4 µM, Alpha Diagnostic; SEQ ID NO.

10), TAT-fused Pep5 (TAT-CFFRGGFFNHNPRYC) (SEQ ID NO. 2) or TAT-fused control peptide (TAT-GGWKWWPGIF) (SEQ ID NO. 15) was used. The peptides were chemically synthesized and their composition was verified by amino acid analysis and mass spectrometry (Sigma Genosys). Amino-terminally FLAG-tagged human p75^{NTR} was cloned into pcDNA3.1 expression plasmid (Invitrogen).

[0400]

10 (Co-precipitation of p75^{NTR} and Rho GDI)
p75^{NTR} precipitated from the transfected 293T cells using anti-FLAG antibody and protein A sepharose, was incubated with recombinant human GST-Rho GDI (Cytoskeleton) or GST-RhoA (Cytoskeleton) in 200 µl buffer (20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 10 mM EDTA, 0.025% Tween20) for 2 hours, and washed. The resultant precipitates were electrophoretically transferred to polyvinylidene difluoride membranes after SDS/PAGE and were immunoblotted with the anti-GST antibody (Sigma). To examine the nucleotide dependency, GST-RhoA was preloaded with the appropriate nucleotide, and EDTA was replaced with 10 mM MgCl₂. Where indicated, Pep5 or the control peptide (GGWKWWPGIF (SEQ ID NO. 15)) was used.

[0401]

25 (Production of Recombinant Proteins)

The p75^{NTR} ICD coding sequence, with or without the deletion, was cloned into the pGEX-5X bacterial expression vectors (Amersham Biosciences) to generate GST-fused proteins from *E. coli*. pGEX-GST-Rho GDI was provided by Dr. Y. Takai. After cell growth to an optical density at 600 nm (OD₆₀₀) of 1.0, 1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) was added to induce protein synthesis, and cells were grown for another 16 hours at 25°C. Fusion proteins were purified

employing glutathione-Sepharose 4B (Amersham Biosciences), and the GST moiety was removed to produce recombinant Rho GDI. Purity of the proteins was determined by SDS-PAGE and the concentration was measured. The deletion mutants of rat p75^{NTR} ICD are from residues 274 to 342, 274 to 351, 274 to 363, 274 to 375, 274 to 390, 274 to 406 and 274 to 425 (Cited Reference 24). Complex formation of GST-p75^{NTR} mutants with Rho GDI was assessed by precipitating the GST-p75^{NTR} mutants.

10 [0402]

(Affinity-precipitation of GTP-RhoA)

Amino-terminally FLAG-tagged human p75^{NTR} or the deletion mutants of p75^{NTR} ICD were cloned into pcDNA3.1 expression plasmid, and were transfected with 293T cells. Cells were lysed in 50 mM Tris (pH 7.5), 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 500 mM NaCl, 10 mM MgCl₂, with leupeptin and aprotinin, each at 10 µg/ml (Cited Reference 25). Cell lysates were clarified by centrifugation at 13,000g at 4°C for 10 min, and the supernatants were incubated with the 20 µg of GST-Rho binding domain of Rhotekin beads (Upstate Biotechnology) at 4°C for 45 min. The beads were washed 4 times with washing buffer (50 mM Tris (pH 7.5) containing 1% Triton X-100, 150 mM NaCl, 10 mM MgCl₂, 10 µg/ml each of leupeptin and aprotinin). Bound Rho proteins were detected by Western blotting using a monoclonal antibody against RhoA (Santa Cruz Biotechnology).

[0403]

(In vitro Nucleotide Exchange Assay)

30 Lipid-modified RhoA was purified from yeast membranes as described (Cited Reference 26). [³H]GDP- or GDP-RhoA complexed with Rho GDI was obtained by first incubating GDP-RhoA with or without [³H]GDP, followed by incubation with

Rho GDI for 30 min, as described previously (Cited Reference 13). The sample, subjected to gel filtration, was equilibrated with 20 mM Tris-HCl (pH 7.5) containing 5 mM MgCl₂, 1 mM dithiothreitol and 0.1% CHAPS. The GDP dissociation and GTP binding assays were carried out by the filter binding method as described previously (Cited Reference 27). In the [³H]GDP dissociation assay, 50 nM of the complex was incubated for 20 min. with various concentrations of GST-fused proteins in a reaction mixture (50 µl) containing 30 mM Tris-HCl (pH 7.5), 5 mM or 0.5 µM MgCl₂, 1 (for a low concentration of Mg) or 10 (for a high concentration of Mg) mM EDTA, 0.1 mM GTP, 1 mM dithiothreitol, 0.12% CHAPS and 0.2 mg/ml bovine serum albumin. In the [³⁵S] GTPγS binding assay, the complex was incubated as described above except that 1 µM [³⁵S] GTPγS was used instead of 0.1 mM GTP. At the indicated time, an aliquot of the reaction sample was removed, and passed through nitrocellulose filters (IPVH000, Millipore). The filters were washed and used for scintillation counting. GST protein or the buffer was used as a control. His-tagged catalytic domain of Dbp was used at the concentration of 90 nM.

[0404]

(Neurite Outgrowth Assay (*In Vitro*))

Dorsal root ganglia were removed from adult mice and dissociated into single cells by incubation with 0.025% trypsin and 0.15% collagenase type 1 (Sigma) for 30 min at 37°C. For cerebellar neurons, the cerebella from two animals were combined in 5 ml of 0.025% trypsin, triturated, and incubated for 10 min at 37°C. DMEM containing 10% FCS was added, and the cells were centrifuged at 800 rpm. Neurons were plated in Sato media (Cited Reference 2) on poly-L-lysine coated chamber slides. For outgrowth assays, plated cells were incubated for 24 hours and were fixed in 4% (wt/vol)

paraformaldehyde, and were immunostained with a monoclonal antibody (TuJ1) recognizing the neuron-specific β -tubulin III protein. Then, the length of the longest neurite or the total process outgrowth for each β -tubulin III-positive neuron was
5 determined. Where indicated, MAG-FC (25 μ g/ml) or the Nogo peptide (4 μ M) was added to the medium after plating. pEF-BOS-myc-Rho GDI plasmid, which was provided by Dr. Yoshimi Takai, or pEGFP plasmid, as a control, was used for the
10 transfection. Twenty four hours after transfection by lipofection, the cells were replated and incubated for 24 hours. To determine the transfected cells, cells were permeabilized and immunostained with the anti-myc antibody (1:1000, Sigma).

[0405]

15 (Nerve Regeneration Effects in Mammal by an Agent Disrupting the Interaction Between a Silencer and/or p75^{NTR} and Rho GDI)

200g male Wistar rats were used. After the ninth thoracic vertebrae laminectomy was performed, the dorsal half of the
20 spinal cord was dissected. A continuous osmotic pump was used to continuously administer either TAT-fused Pep5 (TAT-CFFRGGFFNHNPRYC) or TAT-fused control peptide (TAT-GGWKWWPGIF) to the injured site for 6 weeks (1 mg/weight/day). In this case, the tip of a tube connected to
25 the pump was left in the medullary space. After spinal cord injury, the functional recovery was assessed using the BBB score. The animals were observed on day 7, 14, 21, 28, 35, and 42 after injury. These experiments were carried out using techniques described in Fournier A. E., Takizawa, B. T.,
30 Strittmatter, S. M., J. Neurosci. 2003, 23, 1416-1423.

[0406]

Similar experiments were carried out using anti-p75^{NTR}

antibodies, anti-Rho GDI antibodies, and the extracellular domain of p75^{NTR}.

[0407]

5 These experiments were also carried out using techniques described in Fournier A. E., Takizawa, B. T., Strittmatter, S. M., J. Neurosci. 2003, 23, 1416-1423.

[0408]

10 (Example 1: p75^{NTR} Associates with Rho GDI)

 The present inventors first asked whether the complex of RhoA and Rho GDI associates with the intracellular domain of p75^{NTR}. 293 cells, which express Rho GDI but not p75^{NTR} endogenously, were transfected with FLAG-tagged p75^{NTR} and HA-tagged wild-type RhoA. In the p75^{NTR} precipitates, the anti-Rho GDI antibody revealed the presence of a protein corresponding to Rho GDI (FIG. 1a). As previously shown (Non-patent Document 2), RhoA was included in the complex. The present inventors next examined whether the interaction was strengthened by MAG or Nogo, which have been shown to activate RhoA through a p75^{NTR}-dependent mechanism. N1E-115 cells, which express the Nogo receptor endogenously (data not shown), were transfected with FLAG-tagged p75^{NTR}. The peptide corresponding to residues 31-55 of the extracellular fragment of Nogo (4 μ M) (Reference 9 and soluble MAG-Fc (25 μ g/ml) significantly enhanced the interaction of p75^{NTR} with Rho GDI as well as RhoA (FIG. 1b). In contrast, NGF (100 ng/ml), which inactivates RhoA by p75^{NTR}, abolished the interaction of p75^{NTR} with Rho GDI as well as RhoA. The present inventors previously noted that the interaction of endogenous p75^{NTR} with RhoA could not be observed in neurons (Reference 2). Therefore, the present inventors examined the interaction of endogenous p75^{NTR} with Rho GDI or RhoA using lysates prepared from post-natal

cerebellar neurons from mice (P9). As shown in C of FIG. 6, an association of endogenous p75^{NTR} with RhoA and Rho GDI was observed only after stimulation with MAG or Nogo, suggesting that p75^{NTR} may not be a constitutive activator of RhoA in the
5 cells expressing endogenous p75^{NTR}. These findings demonstrate that Rho GDI in complex with RhoA interacts with p75^{NTR} and that the interaction is strengthened by MAG and Nogo.

[0409]

10 (Example 2: Direct Interaction of p75^{NTR} with Rho GDI)

As RhoA was isolated as a p75^{NTR}-interacting protein by yeast two-hybrid screening, RhoA was suggested to bind directly to p75^{NTR} (Reference 2). However, the fact that endogenous Rho GDI in yeast is active on mammalian Rho family
15 members leaves open an alternative possibility that RhoA in complex with yeast Rho GDI may be associated with p75^{NTR} in the yeast. Therefore, the present inventors next examined the direct physical interaction of p75^{NTR} with Rho GDI or RhoA using purified recombinant proteins. Bacterially produced RhoA, in
20 the GDP-bound, GTP-bound or the nucleotide-depleted state, was incubated with p75^{NTR}, which was precipitated from transfected 293T cells. However, the present inventors observed no interaction between them in any nucleotide state (FIG. 2a). Interestingly, recombinant Rho GDI bound to p75^{NTR}.
25 When prenylated RhoA was complexed with Rho GDI, it associated with p75^{NTR}, suggesting that Rho GDI, but not RhoA, directly complexes with p75^{NTR}.

[0410]

30 The present inventors determined the structural basis of the interaction between Rho GDI and p75^{NTR}. The fifth of the six α -helices of the intracellular domain (ICD) of p75^{NTR} shows significant similarity with the 14-mer peptide mastoparan.

Mastoparan is an amphiphilic component of wasp venom known to activate RhoA. Experiments with the deletion mutant of p75^{NTR} ICD show that the fifth helix is necessary for the interaction of p75^{NTR} with Rho GDI (FIG. 2b). These results suggest that the activation of RhoA by MAG and Nogo may be dependent on the interaction of Rho GDI with the fifth helix of p75^{NTR} ICD. To test this hypothesis more directly, the present inventors employed 293 cells which express no p75^{NTR} endogenously. Affinity precipitation of the GTP-bound form of RhoA revealed that RhoA was activated by the overexpression of full-length p75^{NTR} or p75^{NTR} ICD, as shown previously (Non-patent Document 2). As expected, the deletion mutant that lacks the fifth helix failed to activate RhoA (FIG. 2c), demonstrating that the fifth helix is necessary for the activation of RhoA by p75^{NTR}.

[0411]

(Example 3: Displacement Effect of p75^{NTR} that Releases RhoA from Rho GDI)

Experiments with bacterially expressed p75^{NTR} failed to indicate GDP/GTP exchange activity on recombinant RhoA in in vitro assays (FIG. 3a). These results, in combination with the fact that RhoA does not directly associate with p75^{NTR}, raise the possibility that p75^{NTR} reduces the activity of Rho GDI, thus facilitating the release of RhoA from Rho GDI. This step allows for the activation by guanine nucleotide exchange factors and membrane association of the GTP-bound form of Rho proteins (Cited Reference 8). The present inventors first examined the effect of the interaction of Rho GDI with the helical domain (HD) of p75^{NTR} on its ability to inhibit the GDP/GTP exchange reaction of RhoA at low Mg²⁺ concentrations, as the inhibitory effect of Rho. GDI is more obvious at low Mg²⁺ concentrations (Cited Reference 13). This reaction was

estimated by measuring the dissociation of. [^3H]GDP from [^3H]GDP-RhoA complexed with Rho GDI and the binding of [^{35}S]GTP γ S to GDP-RhoA complexed with Rho GDI. p75^{NTR} HD reduced this Rho GDI activity in a dose-dependent manner (FIG. 3b). Under comparable conditions, glutathione S-transferase (GST) did not affect the Rho GDI activity (FIG. 3b). These results demonstrate that the p75^{NTR} HD has a potency to directly interact with Rho GDI and reduce its ability to inhibit the GDP/GTP exchange reactions of RhoA. The present inventors next examined the effect of p75^{NTR} HD on the Rho GDI ability to inhibit the Db1 stimulated GDP/GTP exchange reaction of RhoA at high Mg²⁺ concentrations. Rho guanine nucleotide exchange factors (Rho GEFs), such as Db1, stimulate the GDP/GTP exchange reaction of. GDP-RhoA free of Rho GDI, but not that of GDP-RhoA complexed with Rho GDI at high Mg²⁺ concentrations (Cited Reference 14). Db1 stimulated the dissociation of GDP from GDP-RhoA (FIG. 3a), but the dissociation of GDP from GDP-RhoA complexed with Rho GDI was markedly reduced (FIG. 3c). However, the dissociation of GDP was restored by p75^{NTR} HD. This inhibitory effect of p75^{NTR} HD on the Rho GDI activity was dose dependent. p75^{NTR} ICD showed the inhibitory effect to the same extent as p75^{NTR} HD (FIG. 3c). These results demonstrate that the interaction of Rho GDI with p75 HD increases its activity in both the RhoGEF-independent and RhoGEF-dependent GDP/GTP exchange reactions of RhoA.

[0412]

As p75^{NTR} has an ability to release RhoA from Rho GDI in vitro, activation of RhoA by MAG and Nogo through p75^{NTR} may be attributable to the activity that releases Rho from Rho GDI. Although MAG, as well as the Nogo peptide, significantly inhibited the neurite outgrowth from post-natal cerebellar neurons, over-expression of Rho GDI abolished these

inhibitory effects (FIG. 3d). These results are consistent with our suggestion that p75^{NTR} acts as a Rho GDI displacement factor.

5 [0413]

(Example 4: The Effect of Peptide Ligand on the Interaction of p75^{NTR} with Rho GDI)

10 All the myelin-derived inhibitors of axonal re-generation identified so far act on neurons through p75^{NTR}, intervening with p75^{NTR} signaling after injury to the central nervous system may alleviate myelin-dependent inhibition of axonal regeneration. Pinpointing the region of Rho GDI association allowed us to develop a strategy to specifically inhibit the function of p75^{NTR}. The specific peptide ligand
15 to the p75^{NTR} HD was previously obtained from a combinatorial library (Reference 15). This ligand is a 15 amino acid residue peptide (Pep5; CFFRGGFFNHNPRYC (SEQ ID NO. 2)) and the binding site was mapped by nuclear magnetic resonance spectroscopy onto a hydrophobic patch framed by helices 5 and 6. Although
20 the sequence of the peptide did not immediately suggest a protein that exists in mammals, the present inventors were interested in the possibility that it may play a role as a silencer that disrupts the recruitment of Rho GDI to p75^{NTR} HD. Surprisingly, the present inventors demonstrated that this
25 peptide might actually function as a silencer. The present inventors first confirmed whether p75^{NTR} associates with Pep5. Glutathione S-transferase-fusion protein containing Pep5 (GST-Pep5) was incubated with lysates prepared from post-natal cerebellum that abundantly express p75^{NTR}. In the GST-Pep5
30 precipitates, the anti-p75^{NTR} antibody revealed the presence of a protein corresponding to p75^{NTR} (FIG. 4a). Then, binding affinity was compared between Pep5 and Rho GDI. p75^{NTR}, immunoprecipitated and purified from the lysates of the

transfected 293T cells, was incubated with 1 μ M GST-Rho GDI and Pep5 at the indicated concentrations (FIG. 4b). Pep5 inhibited the association of p75^{NTR} with Rho GDI dose dependently, but not the control peptide. Therefore, Pep5 has
5 a potential to disrupt the signal mediated by p75^{NTR} *in vitro*. As the peptide ligand must gain entry into the cell if it is to act directly on the p75^{NTR} HD *in vivo*, the present inventors generated Pep5 fused with the amino-terminal 11 amino acid protein transduction domain from the human immunodeficiency
10 virus protein, TAT (TAT-Pep5) (Reference 16). The interaction of p75^{NTR} with Rho GDI induced by MAG-Fc in the dissociated cerebellar neurons was significantly inhibited by TAT-Pep5 in a competitive fashion, but not by TAT-fused control peptide (FIG. 4c). Thus, Pep5 may be used as an inhibitor of Rho GDI
15 association with p75^{NTR}.

[0414]

Similar results were observed in the case of using anti-p75^{NTR} antibody, anti-Rho GDI antibody, and extracellular
20 domain of p75^{NTR} (data not published).

[0415]

(Example 5: Pep5 Silences the Myelin Signal)

Next question the present inventors asked was if Pep5
25 inhibits the effect of MAG or Nogo. The present inventors employed the neurite growth assay to measure the effect of MAG or Nogo. The present inventors used another control peptide derived from rat p75^{NTR} corresponding to residue 368 to 381 of SEQ ID NO. 4. This peptide, at the concentration
30 of 100 nM (FIG. 5b) or 10 μ M (data not shown), had no effect on neurite outgrowth of dorsal root ganglion (DRG) neurons, and it did not influence the action of MAG-Fc (FIG. 5b) or the Nogo peptide (data not shown). However, TAT-Pep5, added

exogenously to cultured neurons at the concentration of 100 nM, abolished their responsiveness to MAG (25 µg/ml) as well as the Nogo peptide (4 µM) (FIG. 5a and b). Post-natal cerebellar neurons were used to examine the effects of Pep5.

5 As observed in DRG neurons, TAT-Pep5 efficiently silenced the inhibitory effect of MAG (25 µg/ml) and the Nogo peptide (4 µM) (FIG. 5c and d). Finally, to show more clearly that the peptide acts as a silencer of p75^{NTR} signaling, the present inventors measured Rho activity by affinity precipitation.

10 As expected, although RhoA was activated 30 min following the addition of MAG-Fc or the Nogo peptide to the post-natal cerebellar neurons, TAT-Pep5 inhibited the activation of RhoA induced by MAG-Fc or the Nogo peptide on these cells (FIG. 5e). These findings strongly suggest that Pep5 inhibits the
15 activation of RhoA through p75^{NTR} by inhibiting the association of Rho GDI with p75^{NTR}.

[0416]

Similar results were observed when experiments were
20 carried out using anti-p75^{NTR} antibodies, anti-Rho GDI antibodies, and the p75^{NTR} extracellular domain.

[0417]

(Example 6: In Vivo Nerve Regeneration Effect of an Agent
25 Disrupting the Interaction Between a Silencer and/or p75^{NTR} and Rho GDI)

200g male Wistar rats were used. After the ninth thoracic vertebrae laminectomy was performed, the dorsal half of the spinal cord was dissected. A continuous osmotic pump was used
30 to continuously administer either TAT-fused Pep5 or TAT-fused control peptide to the injured site. As a result, nerve regeneration was significantly observed when TAT-Pep5 was used, as compared to when the control peptide was used.

[0418]

Similar results were observed when anti-p75^{NTR} antibodies were used.

5

[0419]

(Example 7: Demonstration in Mouse)

Similar experiments were carried out using mice as described above. As a result, nerve regeneration was similarly observed.

10

[0420]

(Example 8: Modified Amino Acid)

Similar experiments were carried out using Pep5 in which alanine was added to the C terminus of the sequence (SEQ ID NO. 2), antibodies for p75 lacking 10 residues of C-terminus, and p75 in which alanine was replaced with valine at amino acid 423 in positions 273-427 of SEQ ID NO. 4. As a result, nerve regeneration was similarly observed.

20

[0421]

For specific circumstances when regeneration is difficult, particularly in an adult body, in the case of nerve-related diseases, disorders and conditions, it has been believed that radical treatment of such are also difficult. However, the effects of the present invention as described above allow for diagnosis which has been conventionally regarded as impossible, and it has been proved that such effects can be applied to treatment. Therefore, it is recognized that the present invention has usefulness which has not been achieved by conventional diagnostic agents and medicaments.

25

30

[0422]

As described above, the present invention has been illustrated by way of preferred embodiments of the present invention, but it is understood that the scope of the invention should be interpreted only depending on the claims. It is understood that patents, patent applications and references cited herein should be incorporated as a reference with regard to the present specification, as if the disclosure per se is specifically described herein.

10 [0423]

(Cited References)

Cited Reference 1. Dechant, G. & Barde, Y. A. The neurotrophin receptor p75 (NTR): novel functions and implications for diseases of the nervous system. Nat Neurosci. 5, 1131-1136
15 (2002).

Cited Reference 2. Yamashita, T., Tucker, K. L. & Barde, Y. A. Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth. Neuron 24, 585-593 (1999).

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Cited Reference 3. Davies, A. M. Neurotrophins: neurotrophic modulation of neurite growth. Curr. Biol. 10, R198-200 (2000).

Cited Reference 4. Schmidt, A. & Hall, A. Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. Genes
25 Dev. 16, 1587-1609 (2002).

Cited Reference 5. Yamashita, T., Higuchi, H. & Tohyama, M. The p75 receptor transduces the signal from myelin-associated glycoprotein to Rho. J. Cell. Biol. 157, 565-570 (2002)
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Cited Reference 6. Wang, K. C. & Kim, J. A., Sivasankaran, R. S., Segal, R. & He, Z. p75 interacts with the Nogo receptor

as a co-receptor for Nogo, MAG and OMgp. Nature 420, 74-78 (2002).

5 Cited Reference 7. Wong, S. T. et al. p75(NTR) and Nogo receptor complex mediates repulsive signaling by myelin-associated glycoprotein. Nat Neurosci. 5, 1302-1308 (2002).

10 Cited Reference 8. Sasaki, T. & Takai, Y. The Rho small G protein family-Rho GDI system as a temporal and spatial determinant for cytoskeletal control. Biochem Biophys Res Commun. 245, 641-645 (1998).

15 Cited Reference 9. Fournier, A. E., GrandPre, T. & Strittmatter, S. M. Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. Nature 409, 341-346 (2001).

20 Cited Reference 10. Masuda, T. et al. Molecular cloning and characterization of yeast rho GDP dissociation inhibitor. J. Biol. Chem. 269, 19713-19718 (1994).

25 Cited Reference 11. Feinstein, D. L. & Larhammar, D. Identification of a conserved protein motif in a group of growth factor receptors. FEBS Lett. 272, 7-11 (1990).

30 Cited Reference 12. Koch, G., Haberman, B., Mohr, C., Just, I. & Aktories, K. Interaction of mastoparan with the low molecular mass GTP-binding proteins rho/rac. FEBS Lett. 291, 336-40 (1991).

Cited Reference 13. Takahashi, K. et al. Direct interaction of the Rho GDP dissociation inhibitor with ez-

rin/radixin/moesin initiates the activation of the Rho small G protein. J Biol Chem. 272, 23371-23375 (1997).

5 Cited Reference 14. Yaku, H., Sasaki, T. & Takai, Y. The Db1 oncogene product as a GDP/GTP exchange protein for the Rho family: its properties in comparison with those of Smg GDS. Biochem Biophys Res Commun. 198, 811-817 (1994).

10 Cited Reference 15. Ilag, L. L. et al. Selection of a peptide ligand to the p75 neurotrophin receptor death domain and determination of its binding sites by NMR. Biochem Biophys Res Commun. 255, 104-109 (1999).

15 Cited Reference 16. Schwarze, S. R., Ho, A., Vocero-Akbani, A. & Dowdy, S. F. In vivo protein transduction: delivery of a biologically active protein into the mouse. Science 285, 1569-1572 (1999).

20 Cited Reference 17. Bentley, C. A. & Lee K, F. p75 is important for axon growth and schwann cell migration during development. J neurosci. 20, 7706-7715 (2000).

25 Cited Reference 18. Walsh, G. S., Krol, K. M., Crutcher, K. A. & Kawaja, M. D. Enhanced neurotrophin-induced axon growth in myelinated portions of the CNS in mice lacking the p75 neurotrophin receptor. J. Neurosci. 19, 4155-4168 (1999).

30 Cited Reference 19. Lee, K. F, Bachman, K., Landis, S. & Jaenisch, R. Dependence on p75 for innervation of some sympathetic targets. Science 263, 1447-1449 (1994).

Cited Reference 20. McQuillen, P. S., DeFreitas, M. F., Zada, G. & Shatz, C. J. A novel role for p75NTR in subplate growth

cone complexity and visual thalamocortical innervation. J Neurosci. 22, 3580-3593 (2000).

5 Cited Reference 21. Del Pozo, M. A. et al. Integrins regulate GTP-Rac localized effector interactions through dissociation of Rho-GDI. Nat Cell Biol. 4, 232-239 (2002).

10 Cited Reference 22. von Schack et al. Complete ablation of the neurotrophin receptor p75NTR causes defects both in the nervous and the vascular system. Nat Neurosci. 4, 977-978 (2001).

15 Cited Reference 23. Lee, K. F. et al. Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. Cell 69. 737-749 (1992).

20 Cited Reference 24. Liepinsh, E., Ilag, L. L., Otting, G. & Ibanez, C. F. NTR structure of the death domain of the p75 neurotrophin receptor. EMBO J. 16, 4999-5005 (1997)

25 Cited Reference 25. Ren, X. D., Kiosses, W. B. & Schwartz, M. A. Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. EMBO J. 18, 578-585 (1999).

30 Cited Reference 26. Forget, M. A., Desrosiers, R. R., Gingras, D. & Beliveau, R. Phosphorylation states of Cdc42 and RhoA regulate their interactions with Rho GTP dissociation inhibitor and their extraction from biological membranes. Biochem. J. 361, 243-54 (2002).

Cited Reference 27. Hart, M. J., Eva, A., Evans, T., Aaronson, S. A. & Cerione, R. A. Catalysis of guanine nucleotide

exchange on the CDC42Hs protein by the dbl oncogene product. Nature 354, 311-314 (1991).

[0424]

By clarifying the relationship between p75^{NTR} and an interaction agent related to neurite outgrowth inhibition, nerve regeneration is provided, and based on such nerve regeneration, pharmaceutical compositions and methods for treating neurological diseases are provided.

(Explanation of Sequence Listing)

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SEQ ID NO. 15: a control peptide used in Examples

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[0426]

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Glu Asp Glu Glu Asp Glu Glu Asp Glu Glu Glu Glu Glu Asp Asp Glu

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Asp Leu Glu Glu Leu Glu Val Leu Glu Arg Lys Pro Ala Ala Gly Leu

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Ser Ala Ala Pro Val Pro Pro Ala Ala Ala Pro Leu Leu Asp Phe Ser

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70

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Ser Asp Ser Val Pro Pro Ala Pro Arg Gly Pro Leu Pro Ala Ala Pro

- 295 -

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SerAla Pro Ser Leu Pro Pro Ala Ala Ala Val Leu Pro Ser Lys Leu

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- 301 -

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[Brief Description of the Drawings]

[Figure 1]

Figure 1 is co-immunoprecipitation of o75^{NTR} and Rho GDI.

30 A) shows co-immunoprecipitation of p75^{NTR} and Rho or RhoA. In the p75^{NTR} immunoprecipitates, the anti-Rho GDI antibody revealed the presenece of a protein corresponding to Rho GDI. B) shows the effects of MAG and Nogo on the interaction of

p75^{NTR} with Rho GDI or RhoA in the transfected N1E-115 cells. Data are mean \pm S.E. Asterisks indicate statistical significance, *; $p < 0.01$ (Student's *t*-test).

5 C) shows co-immunoprecipitation of p75^{NTR} and Rho GDI using lysates prepared from cerebellar neurons. Association was observed in MAG- and Nogo-treated cells.

[Figure 2]

10 Figure 2 shows that p75^{NTR} directly associates with Rho GDI.

a) shows co-precipitation of p75^{NTR} with recombinant GST-Rho GDI or GST-RhoA. Association was examined by Western blot analysis of the precipitates produced with the purified p75^{NTR} and protein A sepharose. The anti-GST antibody revealed the
15 presence of a Rho GDI in the complex.

b) shows co-precipitation of Rho GDI with the deletion mutants of p75^{NTR}. A schematic representation of the constructs for the deleted mutants is shown. The indicated numbers correspond to residues of the mutants.

20 c) shows affinity precipitation of RhoA in the transfected 293T cells. Overexpression of the full-length of p75^{NTR} or p75^{NTR} ICD elicits activation of RhoA, while the mutated p75^{NTR} that lacks the fifth helix fails to activate RhoA.

25 [Figure 3]

Figure 3 shows that p75^{NTR} reduces the Rho GDI activity.

a) shows that p75^{NTR} is not a guanine nucleotide exchange factor for RhoA. The ability of the proteins to induce the dissociation of ³H-labeled GDP from RhoA in 30min was measured.
30 GST protein or the incubation buffer was used as a control. The graph represents the average of relative amount of initial ³H-GDP remaining bound \pm S.E. from three individual experiments. *, $p < 0.01$; (Student's *t*-test).

b) shows that p75^{NTR} HD inhibits the Rho GDI activity *in vitro*. The GDP/GTP exchange reaction of RhoA in complex with Rho GDI was determined in the presence or absence of p75^{NTR} HD. In the [³H] GDP dissociation assay, the dissociation of [³H]GDP from [³H]GDP-RhoA complexed with Rho GDI was assayed by measuring the radioactivity of [³H]GDP bound to RhoA. In the [³⁵S]GTPγS binding assay, the binding of [³⁵S]GTPγS to GDP-RhoA complexed with RhoA was assayed by measuring the activity of [³⁵S]GTPγS bound to RhoA. Closed circle, GST-p75^{NTR} HD; Open square, GST. *, p<0.01; (Student's t-test).

c) shows that p75^{NTR} inhibits the Rho GDI activity. The GDP/GTP exchange reaction of RhoA stimulated with Db1 was incubated with 90nM GST-Db1 and GST-fused proteins at the indicated concentrations. Closed circle, GST-p75^{NTR} HD; Open square, GST; Open triangle, GST-p75^{NTR} ICD. *, p<0.01; (Student's t-test).

d) shows that overexpression of Rho GDI abolishes the effect of MAG and Nogo. The effect of Rho GDI on the neurite outgrowth of dissociated cerebellar neurons was assessed. Left; images of representative cells transiently transfected with the control or Rho GDI plasmid. MAG, MAG-Fc (25 μg/ml); Nogo, the Nogo peptide (4 μM); Rho GDI, cells transfected with myc-tagged Rho GDI. Data are mean ± S.E. An asterisk indicates statistical significance, *; p<0.01 (Student's t-test).

[Figure 4]

Figure 4 shows that Pep5 inhibits interaction of Rho GDI with p75^{NTR}.

a) shows co-precipitation of p75^{NTR} with recombinant GST-Pep5.

b) shows that Pep5 inhibits the binding of p75^{NTR} with Rho GDI dose dependently.

c) shows co-immunoprecipitation of p75^{NTR} and RhoGDI using lysates prepared from cerebellar neurons. The interaction

was diminished by TAT-Pep5.

[Figure 5]

Figure 5 shows that Pep5 silences the inhibitory action
5 of p75^{NTR}.

a) shows that dissociated DRG neurons were incubated for 24
hours with or without the Nogo peptide, and then were
immunostained with monoclonal antibody (TuJ1) recognizing the
neuron-specific β -tubulin III protein. Nogo, the Nogo
10 peptide; Pep5, TAT-Pep5.

b) shows neurite outgrowth of DRG neurons. MAG, MAG-Fc; HD,
the peptide corresponding to the p75^{NTR} HD (residues 368-381);
p75 (+/+), wild type; p75 (-/-), mice carrying a mutation in
the p75^{NTR} gene. Data are mean \pm S.E. Asterisks indicate
15 statistical significance, *; $p < 0.01$ (Student's *t*-test).

c) shows that dissociated cerebellar neurons were incubated
for 24 hours with or without the Nogo peptide.

d) shows neurite outgrowth of cerebellar neurons. Data are
mean \pm S.E. Asterisks indicate statistical significance, *;
20 $p < 0.01$ (Student's *t*-test).

e) shows affinity precipitation of RhoA in cerebellar neurons.
The Nogo peptide (4 μ M) and MAG-Fc (25 μ g/ml) elicit activation
of RhoA, whereas TAT-Pep5 (1 μ M) completely abolishes these
effects.

25 [Figure 6]

Figure 6 shows p75 signal transduction pathway.



2003-092923

- 1 -

[Name of the Document] ABSTRACT

[Abstract]

[Problems]

5 According to the present invention, nerve regeneration is provided, and based on such nerve regeneration, pharmaceutical compositions and methods for treating neurological diseases are provided.

10 [Means for Solving the Problems]

 The above problems have been solved by using a composition comprising Pep5, p75, Rho GDI, MAG, GT1b, p21 and the like, which are related to the p75 signal transduction pathway, or an agent specifically interacting therewith to
15 block or inhibit the p75 signal transduction pathway, thereby stopping inhibition of regeneration leading to resumption of regeneration.

[Selected Figure] None

FIG. 1

A

FLAG-p75	+	+	+	+
HA-RhoA	-	+	+	+
IP	-	-	+	-

← Rho GDI

Precipitation: p75
Detection: Rho GDI

← RhoA

Precipitation: p75
Detection: RhoA

Lysates

Detection: p75

Detection: Rho GDI

B

cont NGF MAG

Precipitation: p75
Detection: Rho GDI

Precipitation: p75
Detection: RhoA

Detection: p75

Detection: Rho GDI

C

Lysates	+	+	+	+
IP	-	+	+	+

Precipitation: p75
Detection: Rho GDI

← Rho GDI

← p75

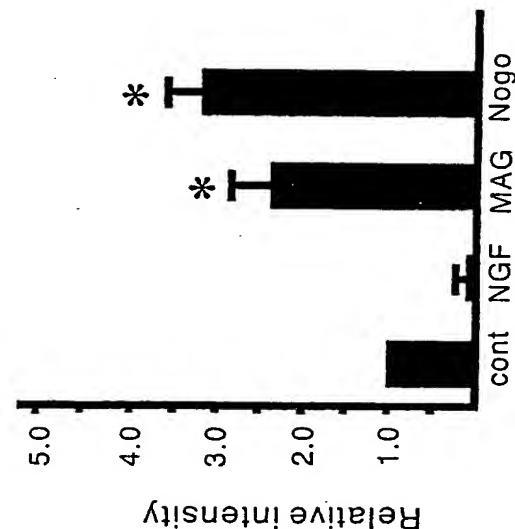


FIG. 2

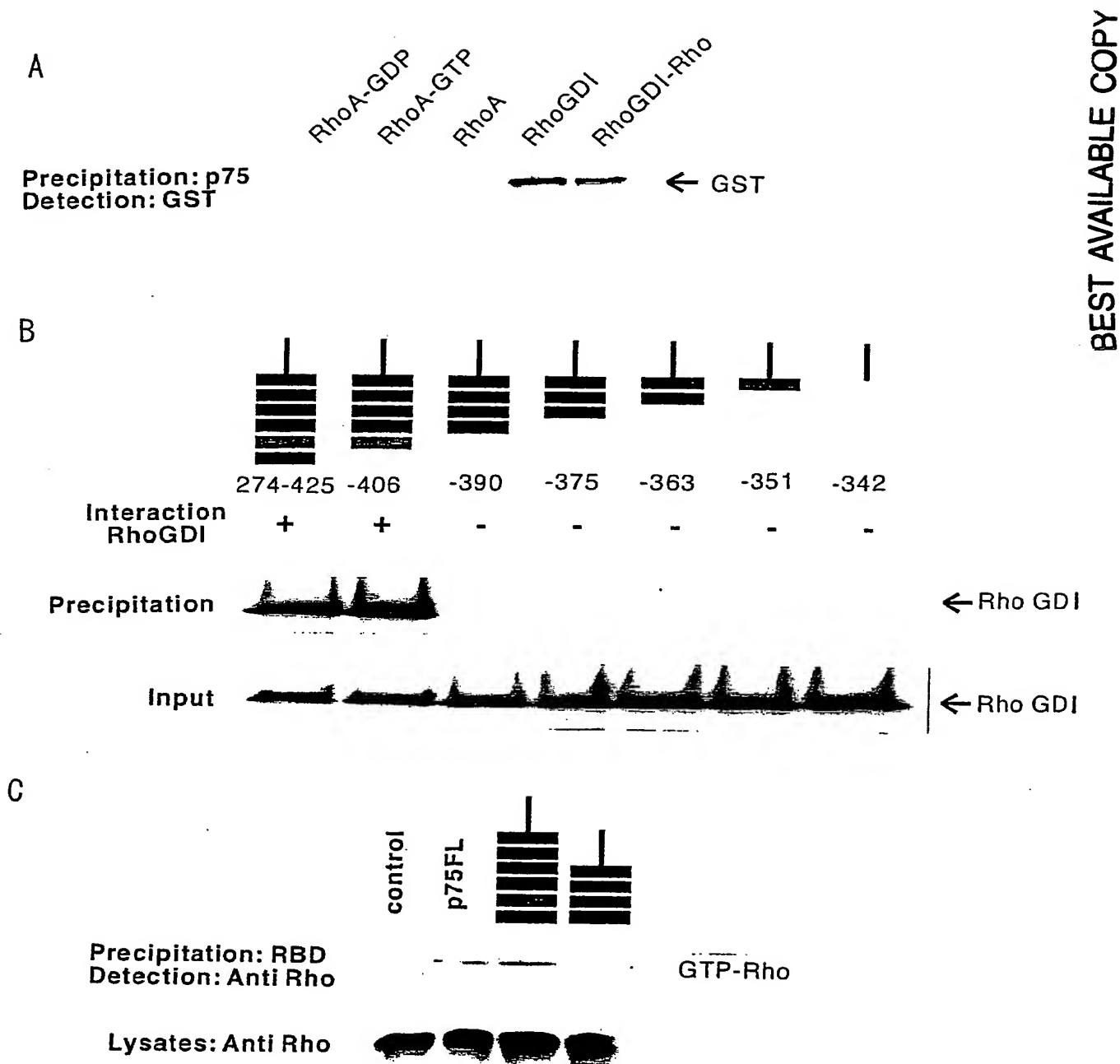


FIG. 3

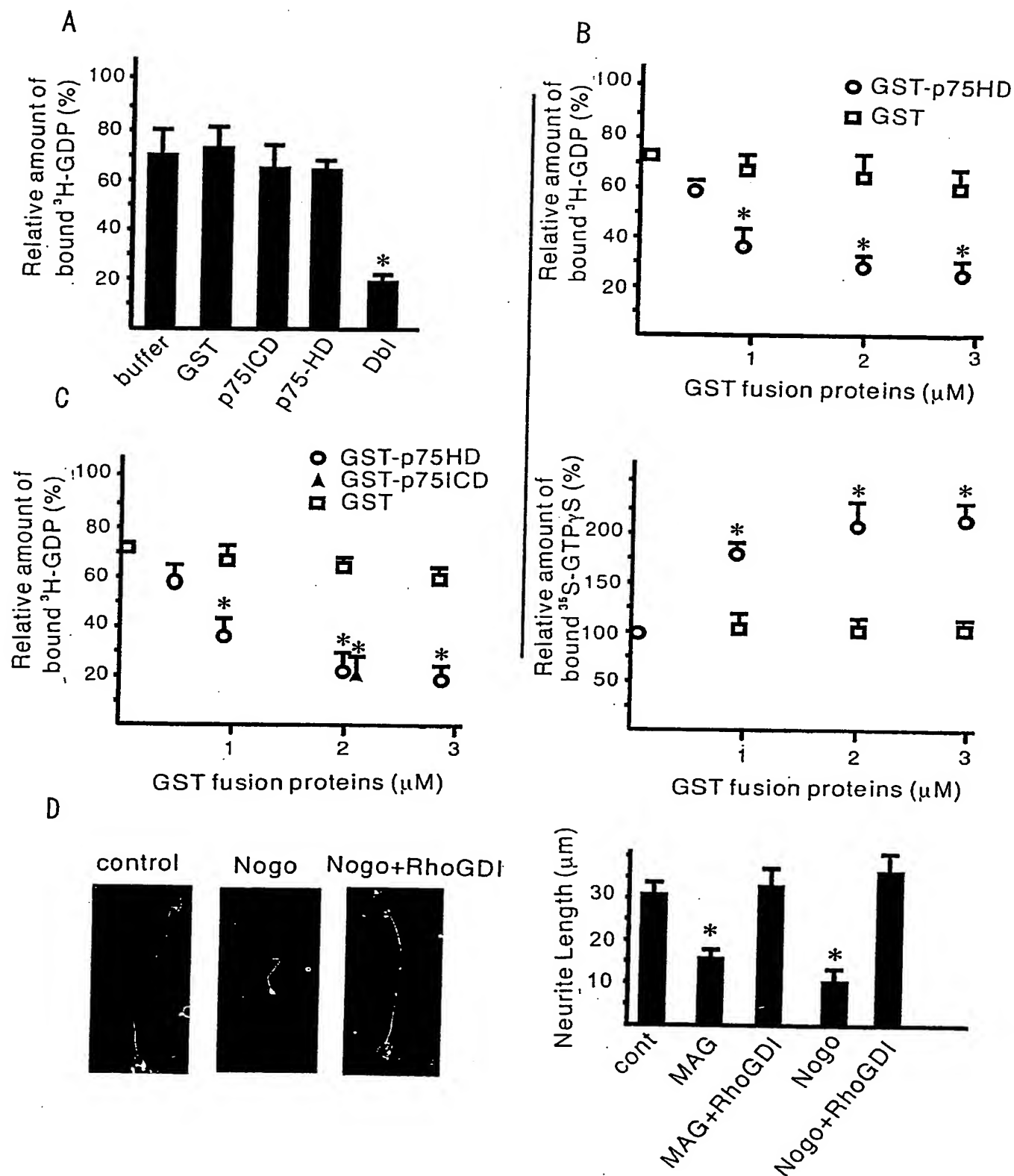
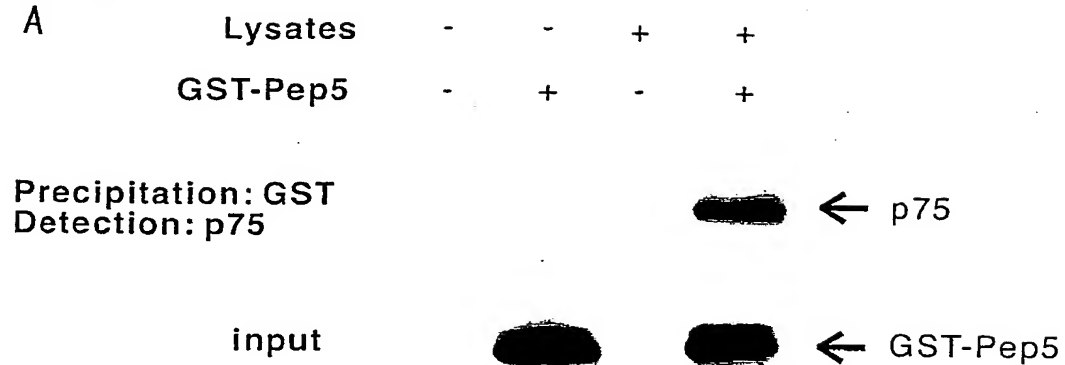
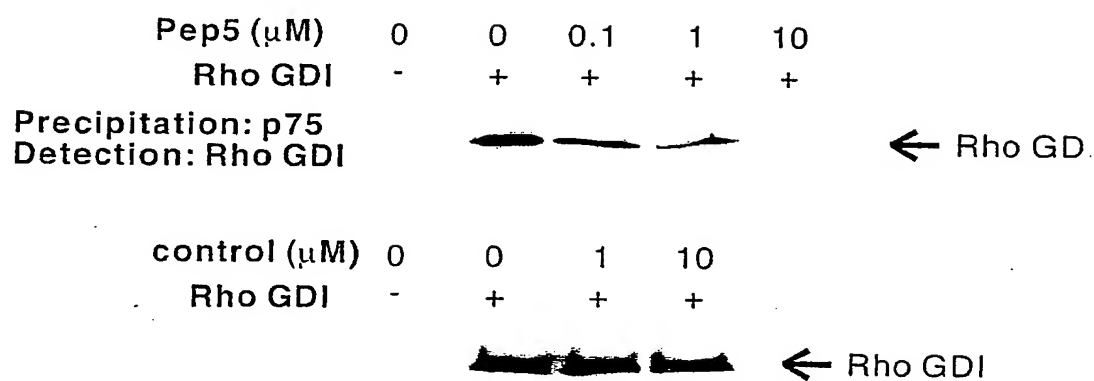
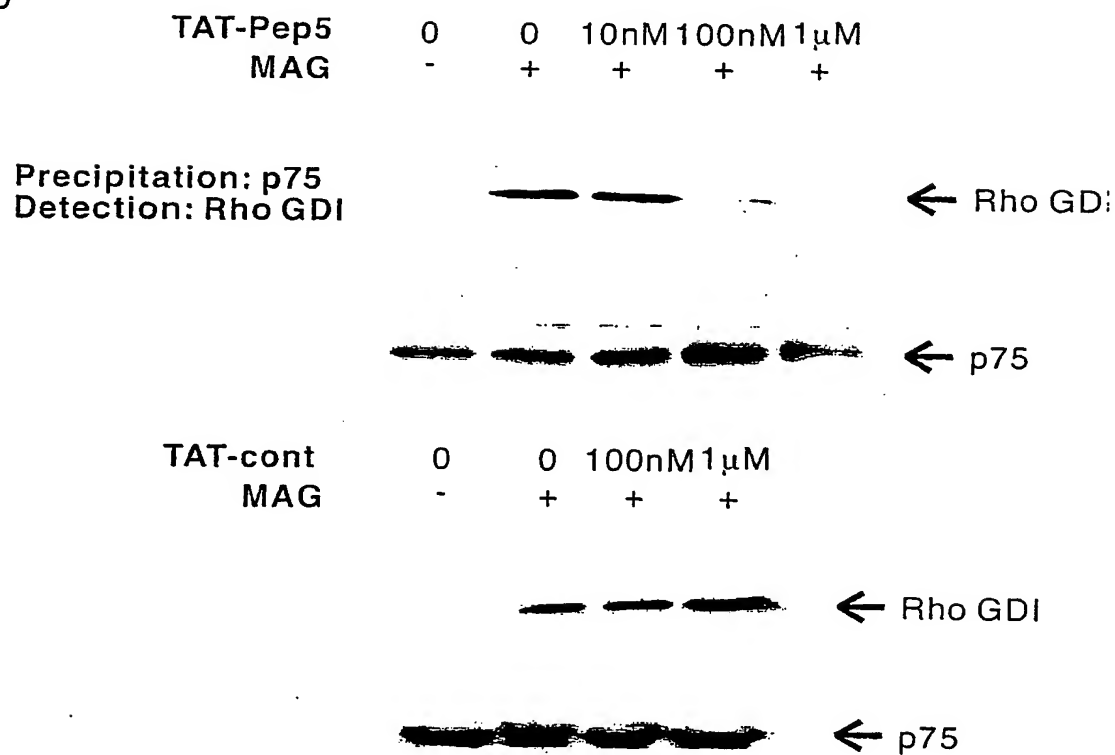


FIG. 4**A****B****C**

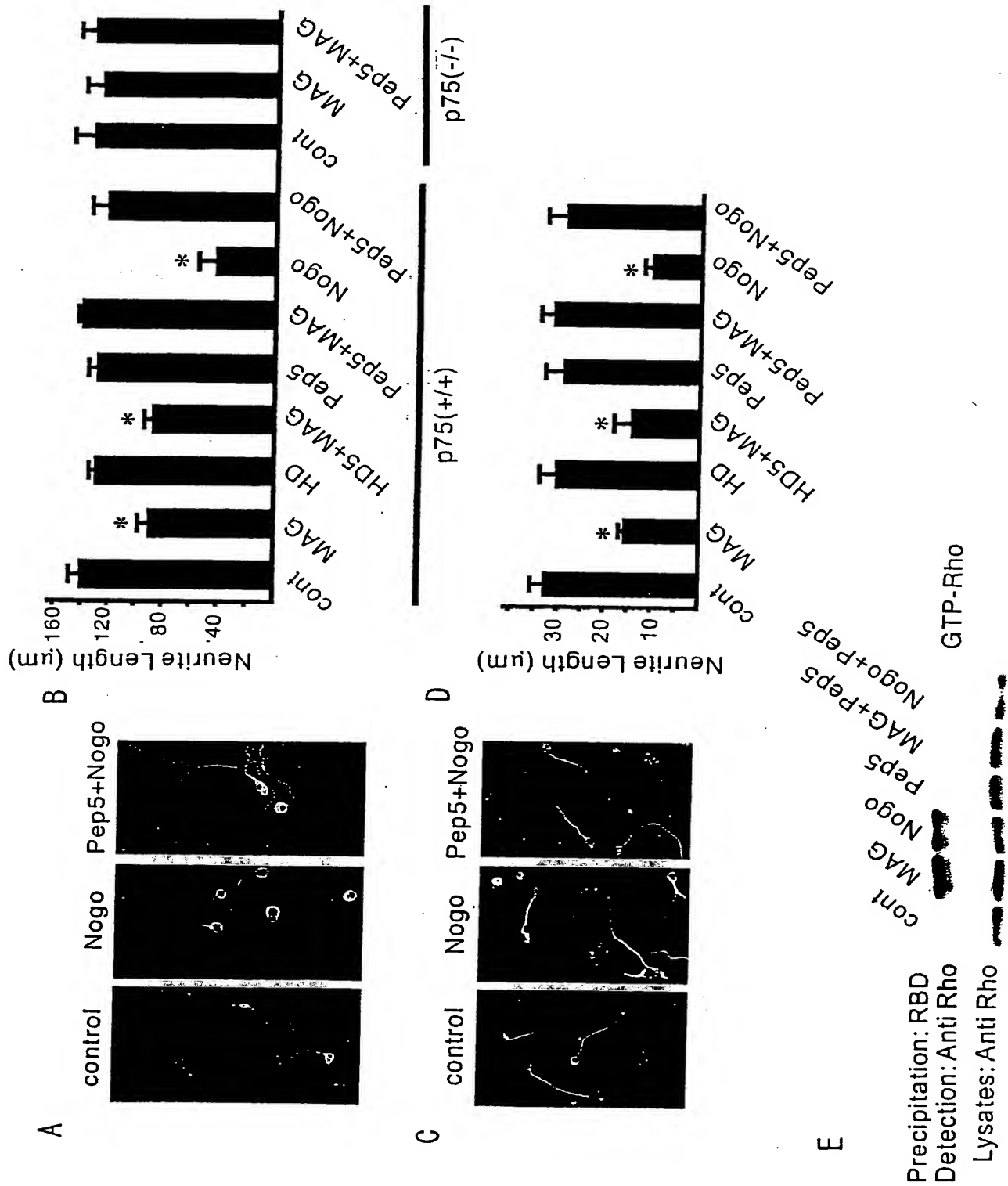


FIG. 6